Striatal dopamine, functional connectivity, and salience processing in health and disease

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Striatal Dopamine, Functional Connectivity, and Salience Processing in Health and Disease

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Acknowledgments

I expect it is not unusual for the end product of a PhD to bear little resemblance to the original outline. After a number of false starts, the fact that there is an end product at all is thanks to the support and generosity of numerous friends and colleagues.

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Abstract

The aim of the current work was twofold. First, to develop methods that allow for the useful integration of distinct methods of examining the human brain, namely positron emission tomography and functional magnetic resonance imaging. Second, to apply these methods to better understand the neurobiological mechanisms underlying the pathways between environmental exposures, schizophrenia, and psychotic symptoms.

The introductory chapter provides an overview of the schizophrenia concept, the neurobiological systems under examination, and the methodologies used. Chapter 2 is a review article that discusses recent developments in our understanding of striatal structure and function, and specifically how striatal dysfunction may underlie many of the symptoms observed in schizophrenia. A specific issue discussed in Chapter 2 is, *where* precisely does striatal dopamine dysfunction occur in schizophrenia? Chapter 3 attempts to answer this using a quantitative approach. A meta-analysis of all studies that have used positron emission tomography (PET) to measure striatal dopamine function in schizophrenia, shows that dopamine dysfunction displays marked spatial variability, and does not occur uniformly across the striatum. I find that dopamine dysfunction occurs predominantly in the associative striatum, refuting the hypothesis that dysfunction within the limbic striatum specifically underlies psychotic symptoms.

Chapter 4 builds on the results of the meta-analysis and uses an integrative PET-fMRI approach to ask whether spatial variability in striatal dopamine function shapes psychopathology in psychosis. I show that dopamine function within striatal regions functionally linked to cortical sensorimotor networks is associated with baseline motor symptoms, while negative and affective symptoms, are linked to dopamine function in striatal regions linked to default mode and cinguloopercular

networks respectively. Chapter 5 again looks at the question of psychopathology, but here I study individuals who have been exposed to risk factors for psychosis, but who have not yet developed a psychiatric disorder. I show that exposure to environmental risk factors is associated with reduced adaptive and increased aberrant salience measures. I also find that these differences in behaviour are related to differences in corticostriatal connectivity

Finally, Chapter 6 uses PET and fMRI to answer an ongoing question regarding neurobiological mechanisms underlying salience processing. Specifically – what is the relationship between mesolimbic dopamine function and the cortical salience network? This is a question of interest because both neural systems share an overlapping role and have been implicated in psychotic disorders. I show that while striatal dopamine *release* capacity is associated with reduced salience network connectivity, striatal dopamine *synthesis* capacity is associated with greater connectivity within the salience network, and that this is particularly the case for 'hub' nodes playing a central role in information processing.

In summary, I show that dopamine dysfunction in schizophrenia does not exist uniformly across the striatum, but is greatest in the associative striatum. The clinical relevance of this is demonstrated by the finding that psychotic symptoms relate to variation in the spatial profile of striatal dopamine dysfunction. In addition, I find that striatal connectivity is altered in individuals exposed to environmental risk factors. Finally, I show how systems known to be crucial to salience processing are linked. Together these findings advance our understanding of schizophrenia by suggesting mechanisms via which striatal and cortical function are linked, how risk factors are associated with neurobiological changes, and how neurobiological abnormalities may shape psychotic symptoms.

In line with the PhD regulations of Kings' College London, it is a PhD incorporating peer-reviewed, published papers

Chapters 2, 4, 5 and 6 have been previously published as the following papers:

Chapter 2: McCutcheon R, Abi-Dargham A, Howes O (2019) Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms *Trends Neurosci* (1-12)

Chapter 3: McCutcheon R, Beck K, Jauhar S, Howes O (2018) Defining the Locus of Dopaminergic Dysfunction in Schizophrenia: A Meta-analysis and Test of the Mesolimbic Hypothesis *Schizophr Bull.* (1-8)

Chapter 5: McCutcheon R, Bloomfield M, Dahoun T, Mehta M, Howes O (2018) Chronic psychosocial stressors are associated with alterations in salience processing and corticostriatal connectivity *Schizophr Res* (1-10)

Chapter 6: McCutcheon R, Nour MM, Dahoun T et al. (2018) Mesolimbic Dopamine Function is Related to Salience Network Connectivity: An Integrative PET and MR Study *Biol Psychiatry* (1-11)

List of Abbreviations

^{11}C-(+)-PHNO	[^{11}C]-(+)-4-propyl-9-hydroxy-naphthoxazine
^{18}F-DOPA	3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine
ACC	Anterior cingulate cortex
ACh	Acetylcholine
aCompCor	Anatomical component based correction
ART	Artifact detection tools
AUD	Auditory network
ASI	Aberrant salience inventory
BOLD	Blood oxygenation level dependent
BP_{ND}	Non-displaceable binding potential
CON	Cingulopercular network
CSF	Cerebrospinal fluid
DAT	Dorsal attention network
DMN	Default mode network
DSC	Dice similarity coefficient
DSM	Diagnostic and Statistical Manual for Mental Disorders
DA	Dopamine
FC	Functional connectivity
fMRI	Functional magnetic resonance imaging
GABA	Gamma-Aminobutyric acid
GWAS	Genome wide association study
ICD	International Classification of Diseases
Ki^{cer}	Influx constant (e.g. of ^{18}F-DOPA)
L-DOPA	L-3,4-dihydroxyphenylalanine
MBq	Megabecquerel
MRI	Magnetic resonance imaging
MSN	Medium Spiny Neuron

NAcc	Nucleus Accumbens
NBS	Network based statistic
omPFC	orbitomedial PFC
PAM	Positive allosteric modulator
PANSS	Positive and Negative Symptom Scale
PET	Positron emission tomography
PFC	Prefrontal cortex
rMRI	Resting state fMRI
ROI	Region of interest
SAT	Salience attribution test
SMN	Sensorimotor network
SN	Substantia Nigra
SPECT	Single photon emission computed tomography
vmPFC	Ventromedial PFC
VTA	Ventral tegmental area

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1. Introduction

Schizophrenia

Schizophrenia accounts for a huge proportion of the global health care burden,[1,2] despite a lifetime prevalence of less than 1%.[3] The fact that a relatively rare disorder is associated with such burden is because it strikes at an early age, and often has an impact sufficiently severe to alter an individual's entire life trajectory.[4] It is a disorder that can be life altering, not only for the person affected, but for families and communities as a whole.

Schizophrenia's place as a central object of study in both medical and neuroscientific research is not only a result of these economic and social costs, but also due to the broader questions that it poses. It is a disease that disrupts the very identity of an individual. The question as to how risk factors that lead to neurobiological alterations are then translated into disruptions of mind, is relevant both for understanding the disease process, and as a window into understanding how the world, brain function, and conscious experience are inexorably linked.

The disease has been recognised throughout history, but the clear portrayal of a distinct syndrome occurs around the end of the 19th century with Emil Kraeplin's detailed descriptions of a dementia praecox.[5] Kraeplin paid particular attention to the chronic course of the illness, which he believed was typically accompanied by an ongoing deterioration in general intellectual capabilities. The illness subsequently became more commonly known as schizophrenia – a term coined by Eugen Bleuler in order to emphasise the splitting between emotional and intellectual functions that he proposed were central to the pathogenesis of the illness.[6] These narrative descriptions, however, were open to interpretation, and it became apparent that the

threshold for diagnosis was poorly defined and thus problematic with regards to diagnostic reliability.[7] The development of the operationalised criteria of the DSM-III addressed this issue and led to the codification of the central features of the disorder that remain in clinical use today.[7]

Although the use of standardised criteria helped to address issues regarding reliability, the validity of the schizophrenia concept has also been questioned.[8] As knowledge regarding the aetiology of schizophrenia has increased, it has become clear that multiple pathways may lead to the development of symptoms that meet diagnostic criteria for the disorder.[9–11] Furthermore, many risk factors,[12] and neurobiological features associated with the illness, are observed across diagnostic categories.[13–15] Attempts have been made to develop a neurobiologically based classification system,[16] but the limitations of current biomarkers mean progress has been limited. It is currently unclear how to cross the tautological impasse inherent in the fact that the diagnostic gold standard remains that of clinical assessment.

Some areas of research that may contribute to advancing past this point are as follows. First, a more detailed understanding of the neurobiological abnormalities seen in psychotic disorders; second, an examination of the links between risk factors and neurobiological alterations; third, investigation of the links between neurobiological alterations and symptoms; and fourth, greater understanding of how different neurobiological systems implicated in psychotic disorders interact.

This thesis reports the results of several experiments undertaken to investigate these ongoing questions regarding the pathways between exposures, neurobiological alterations, and phenotype. Specifically, it examines:

i) The precise location of striatal dopamine dysfunction in schizophrenia

ii) How variability in the location of striatal dopamine dysfunction is linked to symptoms in first-episode psychosis

iii) The relationship between risk factor exposure, neurobiological alterations, and psychopathological sequelae

iv) The relationship between striatal dopamine and cortical connectivity – two systems that have been frequently proposed to underlie the schizophrenia syndrome

In the remainder of this introduction these various topics are briefly discussed, with subsequent in-depth discussion in Chapter 2, as well as in the introductory sections of later chapters.

Risk factors for Schizophrenia

Genetic factors

Initial evidence for a genetic contribution to schizophrenia liability came from twin studies. These demonstrated that concordance rates were greater in monozygotic compared to dizygotic twins, calculating heritability to be around 80%.[17] Recent large scale twin studies using population registers and modern diagnostic criteria are in agreement with these earlier estimates of heritability.[18]

Given this observed heritability, great efforts have been made to characterise the specific genetic underpinnings of schizophrenia. Initial genome sequencing studies, were limited by the number of single nucleotide polymorphisms they could examine, and therefore investigated genes that were a priori hypothesised to play a role in the development of schizophrenia. Recently, however, technological advances and falling costs have made it possible to undertake genome wide association studies (GWAS), allowing an unbiased, data-driven approach in which no candidate genes are

prespecified.[19] These GWAS have found that most of the original candidate genes show no significant association with schizophrenia.[20] In addition, they have provided evidence that in most individuals the genetic contribution is best understood as resulting from multiple common variants of small effect, rather than any single locus contributing large amounts of risk.

The development of GWAS has also enabled the construction of polygenic risk scores. These allow one to calculate the odds ratio for an individual having schizophrenia based on their genetic loading. Individuals with greater genetic loading tend to have more severe symptoms, and may show a poorer response to antipsychotic drug treatment,[21,22] although the clinical utility of this remains to be seen given that the finding has not been consistently replicated, perhaps due to the relatively small effect sizes observed.[23]

The complexity of the pathway between genotype and phenotype is illustrated by the recent finding that there was no relationship between loci implicated in schizophrenia and subcortical brain volumes.[24] This suggests that factors other than genetics may be driving the subcortical volumetric abnormalities in schizophrenia. It is apparently that despite the high degree of heritability environmental factors play a sizable role, given that even among identical twins pairwise concordance is only around 15%.[25]

Environmental Factors

It has become increasingly clear that a range of environmental factors are likely to both have an impact both on schizophrenia risk, and on illness course.[26] For some of these, direct neurobiological links between exposure and neurobiological alteration can be conceived, for example although the precise mechanisms await complete elucidation,[27,28] risk factors such as cannabis use and perinatal complication may have relatively direct neurobiological effects.

For other risk factors such as childhood adversity, migration, and urbanicity it the path to neurobiological consequence is less clear.[29–32] Some progress in understanding potential pathways has been made by conceptualising these risk factors as chronic psychosocial stressors.[9,11,33] The study of individuals exposed to risk factors, but without a diagnosis of mental disorder, has the potential to advance understanding of the pathoaetiological pathway between exposure and disease. The study of disease-free individuals has the advantage that the effect of risk factor exposure is not obscured by the presence of disease, nor the effects of treatment. A number of recent studies have used magnetic resonance imaging to investigate how exposure to environmental risk factors affects brain structure and function. A number of recent studies have used magnetic resonance imaging to investigate how exposure to environmental risk factors affects brain structure and function.[34–38] These studies typically investigate single risk factors at a time. In reality, however, these exposures tend to cluster, and may share common underlying mechanisms.[39–41] In Chapter five below I report the findings of a study examining individuals exposed to multiple environmental risk factors, with a view to investigating what effect such exposure has upon brain function and cognitive mechanisms implicated in psychosis. Specifically, how exposure to chronic psychosocial stress affects corticostriatal functional connectivity, and how this is linked to salience processing.

Dopamine and Brain Networks in Schizophrenia

In addition to our increasing understanding of the pathoaetiology of schizophrenia, significant progress has been made in our knowledge regarding the neurobiological correlates of the disorder.

The Role of Dopamine in Normal Brain Function

Following its identification, dopamine was initially thought to have no biological purpose, and to existing only as an intermediary in the pathway between tyramine and noradrenaline.[42] In the 1950s, this view was challenged by a series of experiments performed by Arvid Carlsson and colleagues. These demonstrated that the movement inhibiting effects of reserpine could be ameliorated by administration of the dopamine precursor L-DOPA, and that this was linked to the synthesis of dopamine (not noradrenaline) within the brain.[43] Furthermore, Carlsson's group found large amounts of dopamine in the brain, in areas of low noradrenaline, supporting the hypothesis that dopamine was an independent neurotransmitter.[44]

Soon after these initial experiments distinct dopamine pathways were identified. Dopamine neurons originating within the midbrain were shown to project to both the striatum (the mesostriatal pathway) and cortex (mesocortical pathway). The mesostriatal pathway includes both mesolimbic projections (from the ventral tegmental area to limbic striatum), and nigrostriatal projections (from the substantia nigra to dorsal striatum).[45] The tuberinfundibular pathway transmits dopamine from the infundibular nucleus of the hypothalamus to the median eminence of the pituitary gland. The striatum receives topographically localised inputs from the cortex, and can be divided into three *functional* subdivisions based on the topography of these cortical afferents. The limbic striatum (also known as ventral striatum) receives inputs from limbic areas of the brain such as the medial prefrontal cortex and amygdala, the associative striatum receives inputs from the dorsolateral

prefrontal cortex, while the sensorimotor striatum receives inputs from sensory and motor cortex.[46,47]

Work by Mawlawi and others defined a set of boundaries for these subdivisions that have subsequently been used in several PET studies.[47] The 'dorsal putamen' here is equivalent to the 'motor striatum' subdivision mentioned in several later chapters, and the 'dorsal caudate' is equivalent to the 'associative striatum'. The boundary between the limbic striatum (inferiorly), dorsal caudate, and dorsal putamen (superiorly) consists of a line between (a) the intersection between the outer edge of the putamen with a vertical line going through the most superior and lateral point of the internal capsule; and (b) the centre of the portion of the anterior commisure transaxial plane overlying the striatum. This line is extended to the internal edge of the caudate. The other boundaries of the ventral striatum are easily distinguished due to its dense signal on MRI. The ventral striatum is sampled from the anterior boundary of the striatum to the level of the anterior commissure coronal plane. Similarly, the dorsal caudate is also sampled from its anterior boundary to the anterior commisure coronal plane. In total, for the dorsal caudate, the sampled region includes the dorsal part of the head of the caudate and the anterior third of the body of the caudate. The dorsal putamen is sampled from its anterior to posterior boundaries. In slices posterior to the anterior commisure plane, the medial boundary of the dorsal putamen is the globus pallidus.

For figures giving an overview of these boundaries, for summary descriptions of the subdivisions, and for more in-depth discussion of dopamine pathways, and in particular the differences between primate and rodent pathways see Chapter 2.

Mesostriatal dopamine neurons have since been shown to signify the discrepancy between expected and actual rewards – a 'reward prediction error'.[48] The concept of a prediction error expands beyond reward, and a prediction error can be considered

to occur in a more general sense whenever a mismatch between prior expectations and reality arises. This more general prediction error is closely related to the concept of 'salience', which refers to the extent to which a stimulus stands out from its surroundings and is thereby able to capture attention. This 'standing out' can occur secondary to various qualities of the stimulus, perceptual salience refers to attributes such as colour and shape, motivational salience refers to a stimulus's ability to engender motivated behaviour (due to its incentivising or aversive properties), and surprise or novelty salience refers to the unexpectedness of a stimulus.[49,50] It has been suggested that dopamine neurons convey information regarding the general salience of environmental stimuli, over and above purely reward related information,[51] and recent findings suggest that aspects of striatal dopamine signalling do not track value but rather factors such as novelty and intensity, and in particular threat related information.[52,53]

Dopamine Function in Schizophrenia

A link between dopamine and schizophrenia came about as a result of a number of complementary pieces of evidence. Preclinical experiments in the early 1960s had suggested that the blockade of monoamine receptors accounted for the clinical effects of antipsychotics, but dopamine was not specifically implicated. It was not until the 1970s, two decades after their introduction, that the clinical effects of antipsychotics were specifically linked to their action on dopamine receptors.[54,55] Additional evidence implicating dopamine in schizophrenia came from findings demonstrating that substances that increased dopaminergic neurotransmission could induce psychotic symptoms in healthy individuals, and worsen symptoms in patients.[56,57] Initial direct evidence for dopaminergic abnormalities in schizophrenia came from post-mortem studies.[58,59] These demonstrated striatal dopaminergic abnormalities in patients with schizophrenia, but findings were inconsistent. A major limitation was also the fact that samples were mostly from individuals that had received extended

periods of antipsychotic treatment, and so it was impossible to determine whether abnormalities were a result of the disease as opposed to treatment.

The findings discussed implicated dopamine in the pathophysiology of schizophrenia, but only indirectly or after death. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) allow for the characterization of the dopamine system in living individuals. This means that dopamine function can be investigated in patients prior to treatment with dopamine antagonists. The consensus established from meta-analysis of these studies is that schizophrenia is associated with increased striatal presynaptic dopamine synthesis and release capacity.[60] The question as to whether post-synaptic abnormalities exist is nuanced, and awaits a definitive answer.[61] Due to the relatively low density of dopamine receptors, and difficulties in generating accurate ligands for D1 receptors imaging of the mesocortical system has been challenging, but recent evidence suggests that cortical dopamine release may be reduced in schizophrenia.[62]

Early theories of dopamine dysfunction schizophrenia posited that hyperdopaminergia within the mesolimbic pathway might be specifically linked to psychotic symptoms. The mesolimbic hypothesis originated with observations that epileptic seizures within limbic areas were associated with psychotic symptoms,[63] and that electrodes implanted in limbic areas were active during periods of psychosis.[64] A link to dopamine specifically, came from animal work linking hyperdopaminergia within the limbic striatum to psychotic-like behaviors, and experiments showing that dopamine antagonism within these same areas was necessary and sufficient for extinguishing these behaviours.[65]

In recent years, the improved resolution of PET cameras has allowed for measurement of dopamine function within functional subdivisions of the striatum. This in turn allows for direct testing of the mesolimbic hypothesis, which I undertake

in Chapter 3. In addition, potential pathophysiological mechanisms linking striatal dopamine dysfunction and psychotic psychopathology are discussed further in Chapter 2. One of these mechanisms is examined in Chapter 4, where I examine whether spatial variability in aberrant striatal dopamine function is mirrored by variability of psychotic symptoms.

Brain Networks

In contrast to the view that schizophrenia results from a localised lesion, it has also been proposed that the disease can be understood as a disorder of connectivity.[66,67] Two neuroimaging techniques have primarily been employed to investigate brain connectivity in schizophrenia. Diffusion weighted imaging allows for the identification of white matter pathways and this has been used to quantify what has been termed *anatomical* or *structural* connectivity. In contrast, *functional connectivity* can be measured using fMRI and refers to a statistical relationship (often a correlation), between the activity of different brain regions.

Studies investigating both anatomical and functional connectivity have demonstrated widespread disruptions of brain networks in schizophrenia.[68–70] Recently a number of studies have highlighted the role aberrant function of a specific network, the salience network, may play in schizophrenia.[14,71–74] In addition, recent meta-analyses synthesising structural and functional imaging data have identified this network as uniquely affected across psychiatric disorders.[13,14] The salience network (also referred to as the cingulo-opercular network), is centred around the anterior insula and dorsal anterior cingulate, and has been proposed to contain subcortical structures such as the limbic striatum and substantia nigra. This network plays a central role in identifying relevant internal and external stimuli, and switching between the default mode network and task positive frontoparietal networks in order to guide behaviour appropriately.[75–78]

Both mesostriatal dopamine neurons and the salience network play overlapping roles in the identification of behaviourally relevant stimuli, and both systems show aberrant functioning in psychotic illness. The relationship between these two systems, however, is not well understood. I attempt to address this in Chapter 6, where PET was used to measure both dopamine synthesis and release capacity, while resting state MRI was used to quantify salience network connectivity.

Methodological Overview

Two methods were used to investigate brain function. PET allows for the in vivo quantification of neurochemical functioning, while resting state MRI allows for the characterisation of functional brain networks.

Positron Emission Tomography

Prior to undertaking a PET scan, it is necessary to manufacture a suitable radiotracer. This involves the labeling of a biologically relevant compound with a radionuclide such as carbon-11 (11C) or fluorine-18 (18F). The labelled compound is subsequently injected intravenously into the participant. The participant is placed in the PET scanner and as the radionuclide undergoes positron emission decay it emits a positron. The positron travels (typically less than 1mm) until it interacts with an electron – annihilating both particles and emitting a pair of gamma rays travelling in opposite directions. The gamma rays are detected by a scintillator within a detection ring and the process of image reconstruction allows the original position of the radionuclide, and thereby the compound of interest, to be imputed.

In the studies reported, two radioligands were employed - 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (18F-DOPA) and [11C]-(+)-4-propyl-9-hydroxy-naphthoxazine (11C-(+)-PHNO). 18F-DOPA PET measures the rate constant (Kicer) for 18F-DOPA uptake, transport into synaptic vesicles, and its conversion

into 18F-dopamine, thus providing a measure of dopamine synthesis capacity.[79] ^{11}C-(+)-PHNO is a D2/3 agonist and provides a means to quantify dopamine receptor density within the striatum. A placebo scan gives a measure of baseline D2/3R availability (non-displaceable binding potential, BPND), while a scan following dexamphetamine administration allows quantification of the change in BPND due to competition from increased synaptic dopamine concentrations. The percentage reduction in D2/3R availability between placebo and dexamphetamine scans thus provides an index of dopamine release capacity.

PET is used in most of the chapters in this thesis. Chapter 3 is a meta-analysis of PET studies examining the dopamine system in schizophrenia, Chapter 4 uses 18F-DOPA PET to measure striatal dopamine function in individuals with a first episode psychosis, while Chapter 6 uses both 18F-DOPA and 11C-PHNO to relate striatal dopamine function to salience network connectivity.

Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging is provides a measure of brain activity. It is an indirect method in that it primarily measures blood flow, from which neural activity is estimated. Oxygenated haemoglobin is a diamagnetic molecule whereas deoxygenated haemoglobin is paramagnetic. Neural activity brings with it a demand for glucose, and this demand is met by increased bloodflow to the active area. The increased bloodflow is composed of blood that contains a greater proportion of oxygenated haemoglobin – leading to a measurable change in the magnetic properties of the region in question that can be detected using MRI.[80]

Initial studies focused on using this technique to identify regions of the brain that increased in activity when performing a task. Work by Biswal, Raichle and others began to use functional MRI to systematically characterise the activity of the brain in conditions where an explicit task was not being performed.[81,82] It has subsequently

been shown that activity within networks of brain regions is temporally correlated even in the absence of explicit external demands,[83] and furthermore that these networks underlie human cognition and behaviour.[84,85]

Resting state MRI is employed to study functional brain connections in three chapters of the present thesis. Chapter 4 uses corticostriatal connectivity measures to parcellate the striatum based on its cortical connections, and to see how dopamine function within these parcels relates to psychotic symptoms. Chapter 5 employs a seed to voxel approach to investigate corticostriatal connectivity and examines how this relates to exposure to environmental risk factors for psychosis, while Chapter 6 uses a graph theoretical approach to investigate the network structure of the salience network and how this relates to striatal dopamine function.

Aims and Hypotheses

The overall intention of the work was to use a multimodal approach to better understand the pathoaetiological processes underlying psychotic symptomatology. More specifically, I aimed to achieve the following:

- Chapter 2: To characterize the current state of scientific knowledge regarding striatal structure and function, in terms of both normal physiology, and the dysfunction observed in schizophrenia.

- Chapter 3: To synthesise the results of all PET studies that have measured striatal presynaptic dopamine function in schizophrenia, and determine where within the striatum dopaminergic dysfunction is greatest. We aimed to test the mesolimbic hypothesis of schizophrenia – that dopaminergic hyperactivity of the limbic striatum underlies psychosis.

i. Hypothesis: Presynaptic dopamine function will be greater in individuals with schizophrenia compared to healthy controls.

ii. Hypothesis: This presynaptic hyperdopaminergia will not occur uniformly across the striatum but will be greater in certain subdivisions compared to others. Specifically, I aimed to test the mesolimbic hypothesis of schizophrenia – that dopaminergic dysfunction is greatest in the limbic striatum.

- Chapter 4: To use PET to measure dopamine synthesis capacity in individuals with schizophrenia, and resting state fMRI to parcellate the striatum of these individuals on the basis of corticostriatal connectivity patterns. On the basis of this, to then investigate whether dopamine dysfunction within specific striatal regions is linked to the specific symptoms one would predict on the basis of the connected cortical area.

  i. Hypothesis: Specific symptoms will be associated with dopamine dysfunction in specific striatal subregions that show preferential connectivity with functionally-relevant cortical regions. I focus on auditory hallucinations and motor symptoms as these are symptoms that have a priori links to well circumscribed (auditory and motor) cortical areas. Specifically, we predict that both baseline severity, and change (following antipsychotic treatment) in severity, of hallucinations and of motor symptoms would correlate with dopamine synthesis capacity in striatal regions preferentially connected to auditory and motor cortex, respectively.

  ii. I undertook an exploratory analysis investigating whether any notable relationships occurred between dopamine dysfunction in other cortical connectivity defined striatal subregions and other symptom clusters.

  iii. We compared our connectivity defined striatal parcellation with published atlas-defined subdivisions, in order to examine whether an individualised data-driven connectivity-based method is able to provide additional information over an atlas-based approach.

- Chapter 5: To investigate the cognitive and neurobiological correlates of exposure to chronic psychosocial stressors that are established environmental risk factors for psychosis. To do this, I employed resting state MRI and a behavioural task (the salience attribution task) to test the following hypotheses:

  i. Hypothesis: Individuals with a history of high exposure to chronic psychosocial stressors will display increased aberrant, and reduced adaptive, salience scores compared to individuals with a history of low exposure.

  ii. Hypothesis: Individuals with a history of high exposure to chronic psychosocial stressors will display altered corticostriatal functional connectivity compared to individuals with a history of low exposure.

  iii. Hypothesis: Alteration in corticostriatal connectivity will be related to alterations in salience processing.

- Chapter 6: To examine the relationship between two key salience processing systems: the cortical salience network and the mesolimbic dopamine system. We used resting state fMRI to characterise the salience network in two separate cohorts – one that had received an 18F-DOPA PET scan (to measure dopamine synthesis capacity), and the other that had received an 11C-PHNO scan before and after amphetamine administration (to measure dopamine release capacity).

  i. Hypothesis: Individuals with greater striatal dopamine synthesis and release capacity will show greater connectivity within the salience network, and, because of the reciprocal relationship between salience and default mode networks, weaker connectivity within the default mode network.

  ii. Hypothesis: In addition, I identified within these networks, regions that played the most important role in information processing ("hub nodes"). Hubs support the rapid integration of information across a complex system and as such can be considered an optimal target via which a network input may efficiently maximize its influence in a coordinated fashion. I therefore

hypothesized that there would not be a uniform association between dopamine function and connectivity but that hub nodes would show the strongest association with dopamine.

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2. Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms

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The mesolimbic hypothesis that aberrant functioning of midbrain dopamine projections to limbic regions causes psychotic symptoms has been a central dogma of schizophrenia for decades. Recently, however, advances in neuroimaging techniques have led to the unanticipated finding that dopaminergic dysfunction in schizophrenia is greatest within nigrostriatal pathways, implicating the dorsal striatum in the pathophysiology of the illness and calling into question the mesolimbic theory. At the same time our knowledge of striatal anatomy and function has progressed, suggesting new mechanisms via which striatal dysfunction may contribute to the symptoms of schizophrenia. This review draws together these developments, to explore what they mean for our understanding of the disorder's pathophysiology, clinical manifestations, and treatment.

Highlights

Techniques for characterising the mesostriatal dopamine system, both in humans and animal models, have advanced significantly over the past decade

In vivo imaging studies in schizophrenia patients demonstrate that dopaminergic dysfunction in schizophrenia is greatest in nigrostriatal as opposed to mesolimbic pathways

Better understanding of striatal structure and function has enhanced our insight into the neurobiological basis of psychotic symptoms

The role of other neurotransmitters in modulating striatal dopamine function merits further exploration, and modulating these neurotransmitter systems has potential to offer new therapeutic strategies

Schizophrenia and the striatum

Schizophrenia is a syndrome consisting of positive (such as delusions and hallucinations), negative (including flattened affect and lack of motivation), and cognitive symptoms. Dysregulated dopaminergic modulation of striatal function is fundamental to many models that seek to explain the mechanisms underlying the symptoms of schizophrenia[1–4]. Furthermore, all licensed pharmacological treatments for schizophrenia affect the dopamine system, and while several atypical antipsychotics have been proposed to act via alternative non–dopaminergic

mechanisms, such as the serotonergic system, it is still the case that they all bind to dopamine receptors, and there is no clear relationship between efficacy and serotonergic effects[5].

In this paper, we review how advances in neuroscientific methods have improved our understanding of striatal structure and function. We then examine the evidence regarding striatal dysfunction in schizophrenia, and discuss how recent findings suggest a re-evaluation of prior hypotheses may be required. Finally, we ask what these developments mean for our understanding of the disorder's clinical manifestations and treatment.

Striatal Structure and Function

Striatal Connectivity

The striatum is an integral part of the cortico-basal ganglia circuitry. Extensive work mapping its pathways, as summarized below, suggests that it acts as an integrative hub for information processing in the brain.

Initial primate research aimed at mapping striatal connections involved lesioning cortical areas and recording the location of subsequent

Glossary

Amphetamine sensitisation: Repetitive administration of amphetamine leading to progressively greater amphetamine induced dopamine release.
Associative striatum: Precommissural dorsal caudate, postcommissural caudate, and precommissural dorsal putamen. Receives afferent connections from the dorsolateral prefrontal cortex. Homologous to the rodent dorsomedial striatum.
Clinical high risk (CHR): Individuals experiencing intermittent or attenuated psychotic symptoms, below the level at which a psychotic disorder would be diagnosed.
Direct Pathway: Striatal output pathway, in which D1 MSNs project directly from the striatum to the output nuclei of the basal ganglia.
Diffusion Tensor Imaging: Measurement of water diffusion patterns to infer the white matter structure of the brain and map anatomical connectivity between regions.
Efference copy: An internally generated replica of an outgoing motor signal, that signals that the subsequent motor act is self-generated and thereby dampens sensory perceptions occurring as a result of that act.
Inappropriate affect: An emotional expression not in keeping with the circumstances that provoked it.
Indirect pathway: Striatal output pathway, in which D2 MSNs project from the striatum to the output nuclei of the basal ganglia indirectly via the pallidum.
Limbic striatum: Equivalent to the ventral striatum. Receives afferent projections from limbic areas: vmPFC, OFC, dACC and medial temporal lobe

striatal degeneration. Later work used retrograde tracers injected into the striatum to determine both cortical and midbrain connections [6]. Striato-cortical connections were shown to run in three parallel, and relatively well segregated pathways, that effectively parcellated the striatum into **limbic** (see glossary), **associative**, and **sensorimotor** functional subdivisions based on their specific inputs and outputs (Figure 1A)[7]. At the time, it was thought that these corticostriatal loops operated in parallel with minimal cross talk, an idea referred to as the 'parallel processing' model. Subsequent studies, however, suggested that in addition to these parallel loops, there are projections from one loop to another [8], which promote 'information funnelling' from the ventral to the dorsal striatum (Figure 1A).

Medium Spiny Neuron (MSN): GABAergic projection neurons of the striatum. Typically classified as either D1 type of the direct pathway, and D2 type of the indirect pathway.
Nucleus Accumbens (NAcc): Main component of the ventral striatum.
Passivity phenomena: Symptom of schizophrenia in which one feels under external influence and no longer in control of one's movements or thoughts.
Positive psychotic symptoms: Symptoms such as hallucinations, delusions, and disorganised thought and behaviour**.**
Positron Emission Tomography (PET): Imaging technique in which a radioactive atom is attached to a biologically active molecule. Positrons emitted by this molecule produce gamma rays which are detected to allow visualisation of its anatomical distribution.
Resting state functional magnetic resonance imaging: Brain activity is measured in the absence of any explicit task, and correlations between spontaneous fluctuations allow for the inference of functional connectivity between regions.
Salience: The quality of an item that causes it to stand out from its environment.
Sensorimotor striatum: Post commissural putamen. Receives afferent projections from motor and premotor cortical areas. Homologous to dorsolateral striatum in rodents.
Thought echo: A form of auditory hallucination in which an individual perceives their thoughts being spoken aloud as an external stimulus.

Recent methodological advances have further refined our understanding of corticostriatal architecture [9,10]. These advances underscore the lack of a strict 1:1 topographic mapping, and indicate that corticostriatal pathways overlap. Based on its exceptional degree of input heterogeneity, the associative striatum has been highlighted as an information processing hub [11]. Furthermore, cluster analysis of corticostriatal input patterns has shown that in addition to the three subdivisions discussed above, a fourth subdivision is apparent in the tail of the

striatum, its most caudal part [11]. This region receives cortical input from a range of areas including limbic and auditory cortex, and is distinct in its composition, consisting almost exclusively of D1 expressing **medium spiny neurons** (MSNs) [12].

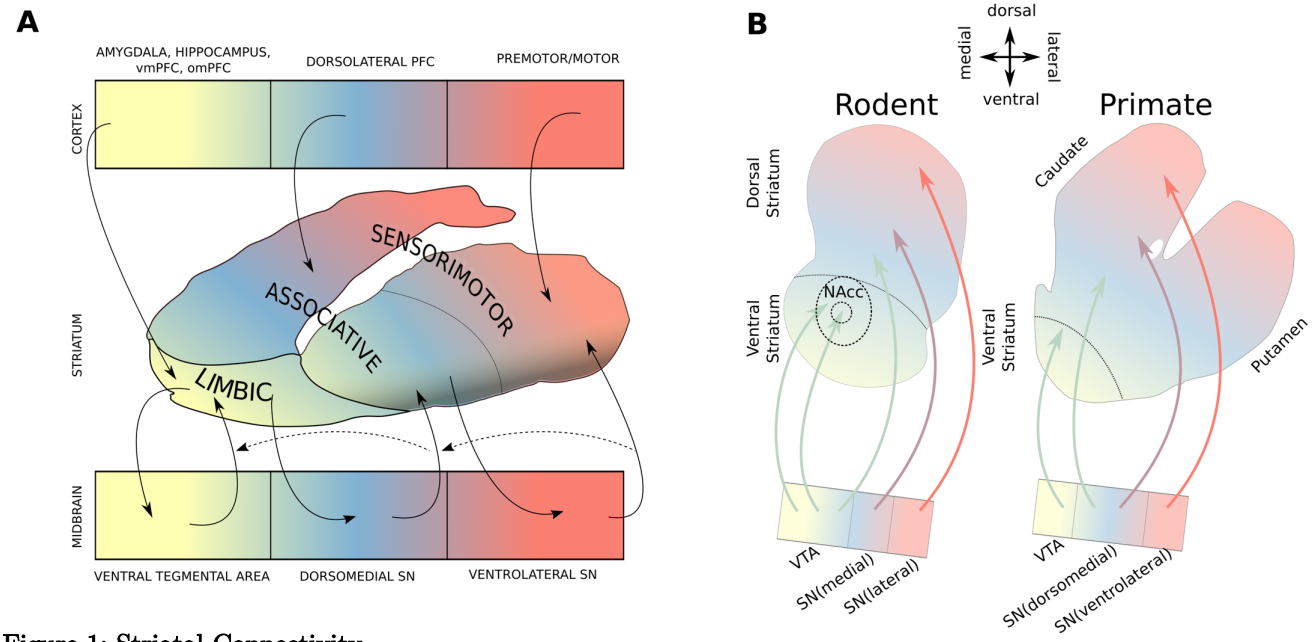

Figure 1: Striatal Connectivity

A) Summary of primate tracing studies mapping connections between cortex (top row), striatum (middle) and midbrain (bottom).

The primate striatum can be divided into nucleus accumbens, olfactory tubercle, caudate nucleus and putamen (for simplicity, not indicated in the figure). The division between caudate and putamen, however, has relatively little biological relevance [6]. Tract tracing studies showed that striato-cortical connections run in three parallel pathways. Motor areas project to the caudal putamen [117]; dorsolateral prefrontal cortex to caudate and rostral putamen [118]; and limbic areas to the ventral striatum [119]. These subdivisions were termed the **sensorimotor**, **associative**, and **limbic striatum**. Subsequent research used retrograde tracers injected into striatum to determine midbrain connections [6,8]. This showed that ventral tegmental area and medial substantia nigra project primarily to limbic striatum, while central/ventrolateral parts of substantia nigra project to associative and sensorimotor striatum. The striatum in turn has efferents projecting back to the midbrain. In addition to these reciprocal connections, 'feed forward' striato-nigro-striatal connections allow information to pass along the striatum from limbic to motor regions via the associative striatum [8,112,120].

B) Summary of rodent-primate differences in mesostriatal connectivity.

In rodents, the ventral striatum is proportionally larger than in primates. The NAcc shell is innervated by the medial VTA, the NAcc core by the central VTA, whereas the lateral VTA innervates a region homologous to the associative striatum; the substantia nigra also has some connections to the associative region in addition to the more dorsal regions of the striatum. In primates, the VTA is proportionally smaller; it innervates the ventral striatum, whereas the dorsal tier substantia nigra innervates the associative striatum, and the ventral tier innervates the sensorimotor striatum (for a more detailed review of differences between primates and rodents see [14,121]).

omPFC – orbitomedial PFC, PFC – Prefrontal cortex, SN – Substantia nigra, vmPFC – ventromedial PFC, VTA – Ventral tegmental area

In addition to its widespread cortical connectivity, the striatum has extensive bidirectional connections to the midbrain (Figure 1A). The development of the CLARITY tissue preparation method (which enables lipid removal, while preserving tissue structure), has allowed for in depth examination of meso-striatal connectivity, and has identified projections from the dorsolateral to dorsomedial projecting dopamine neurons, suggesting a novel pathway for lateral to medial information flow, in addition to previously identified medial to lateral routes (Figure 1A) [13].

Many of these more recent findings on striatal connectivity have been demonstrated only in rodents to date, and interspecies differences must be borne in mind when seeking to draw parallels with primate and human anatomy (Figure 1B) [14]. Human neuroimaging studies have, however, produced findings that are in keeping with some of the pathways discussed above. **Resting state functional magnetic resonance imaging** [15] and **diffusion tensor imaging** [16] studies support the division of the striatum into functional subdivisions. Moreover, compared to anatomical divisions, these functional subdivisions display greater homogeneity in terms of dopamine release [16], highlighting their relevance for understanding striatal function. Likewise, the preclinical finding that the associative striatum acts as an integrative hub via the convergence of multiple distal cortical inputs, is consistent with human studies [17–19].

In summary, it appears that in addition to well-established parallel pathways, there also exists, across species, a high degree of pathway crossing and information funnelling, with regions in the associative striatum acting as integrative hubs. Furthermore, there appear to be a variety of pathways allowing for bidirectional information transfer across the striatum.

Striatal Neurochemistry

Recent advances have refined understanding of striatal neurochemistry. In this section, we consider these findings and their implications for mechanisms by which abnormalities of non-dopaminergic neurotransmitter systems may contribute to dopaminergic dysfunction. We also discuss their relevance for developing new treatment approaches to normalise striatal dopamine function and possibly treat schizophrenia without requiring dopamine D2/3 receptor blockade.

Most dopamine neurons have the potential to release GABA as a co-transmitter, and a smaller proportion co-release glutamate. This co-transmission varies across the striatum, and can moderate the reciprocal relationship between dopaminergic and cholinergic neurons [20]. Specifically, in the dorsal striatum, dopamine neurons do not co-release glutamate, and the firing of dopamine neurons in this region is accompanied by pauses in cholinergic interneuron firing secondary to dopamine D2 receptor and GABA signalling. In the ventral striatum, by contrast, a burst-pause occurs secondary to glutamate co-transmission [20]. In addition to these functional differences across the striatum, dopamine receptors *themselves* show fundamentally different responses to dopamine, dependent on their striatal location. D2 receptors in the accumbens show both greater sensitivity to dopamine and a slower postsynaptic current compared to those in dorsal regions [21]. This is not secondary to differences in D2/3 ratios, but rather appears to result from differences in Gα subunits [21].

Corticostriatal neurons synapse upon cholinergic interneurons, which in turn modulate dopamine neurons via nicotinic receptors situated on these dopamine neurons, thereby mediating the corticostriatal control of striatal dopamine release [22]. It is also these cholinergic interneurons that drive GABA release from dopaminergic neurons [23]. Muscarinic regulation of striatal dopamine function has also been shown, although there is no evidence that dopamine neurons in the striatum display muscarinic receptors [24], and it appears this modulation occurs

secondary to a variety of mechanisms, including autoreceptors on cholinergic terminals that inhibit acetylcholine release [25], endocannabinoid signalling pathways [26], and modulation of MSN projections to the substantia nigra [27]. In the dorsal striatum both M2 and M4 receptors are needed for this muscarinic modulation of dopamine release, whereas in the ventral striatum only M4 receptors are required [24].

Of relevance to the treatment of schizophrenia, Kharkwal et al. demonstrated that the extra pyramidal side effects of antipsychotic medications may primarily result from the blockade of D2 receptors on cholinergic interneurons [28]. Blockade of D2 receptors was shown to increase the firing of D2 expressing **indirect pathway** MSNs, both due to the direct effect on these neurons, but also as a result of increased acetylcholine mediated activation of M1 receptors on the MSNs, occurring as a result of D2 blockade on cholinergic interneurons (Figure 2A)[28]. The authors suggest that this mechanism may have the potential to minimise movement side effects, and this is supported by the finding that the antipsychotic with the lowest risk of these is clozapine. While clozapine's favourable profile in this domain may also result from its low affinity for the D2 receptor and low D2 occupancy at clinically therapeutic doses, it also displays significant antagonism at the M1 receptor, which these findings suggest may contribute significantly to its lack of significant movement side effects.

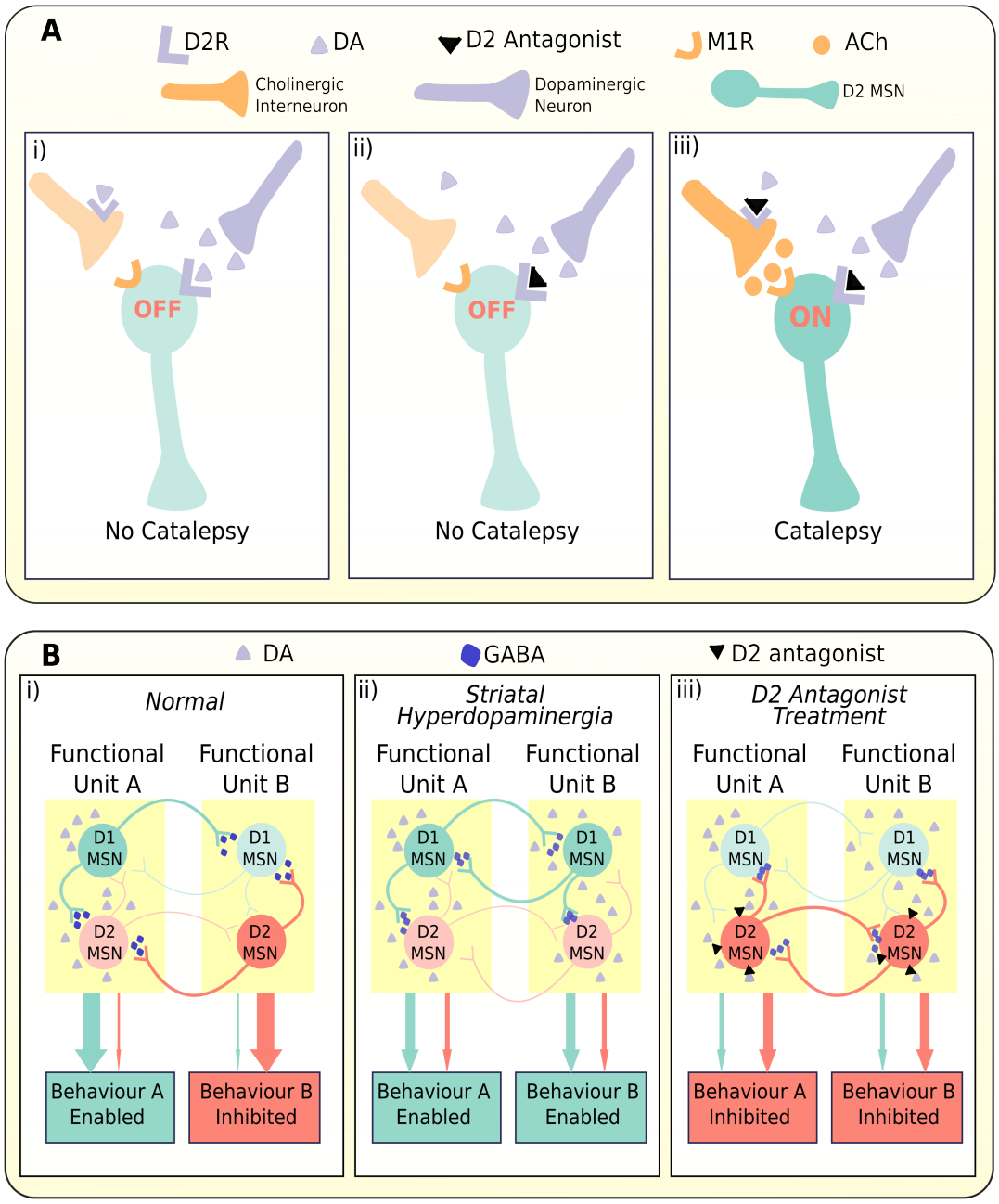

Figure 2. Striatal neurochemistry and neurotransmission

A) The role of cholinergic interneurons in mediating extrapyramidal side effects (see Kharkwal et al [28])
(i)In wild type mice, D2Rs mediate inhibitory actions both by directly reducing firing of the indirect pathway MSN, and by reducing firing of cholinergic interneuron (ii) In knockout mice without D2R on cholinergic interneurons, D2 antagonism of the MSN by itself is insufficient to induce catalepsy (iii) Activating, in addition, M1Rs, results in catalepsy, which occurs due to increased firing of the cholinergic interneuron secondary to D2 antagonism.

B) The relationship between striatal dopamine, lateral inhibition and behavioral selection(see Burke et al [35]). The figure illustrates three scenarios, based on a hypothetical pair of 'functional units' (A, and B) each controlling a specific behavioural outcome (behavior A and B, respectively).
(i) Localized dopaminergic signaling in functional unit 'A' activates D1 direct pathway MSNs, while suppressing D2 indirect pathway MSNs, enabling the execution of desired behavior 'A'. GABAergic lateral inhibition suppresses competing behavior coded for by functional unit 'B'. (ii) Spatially disorganized dopaminergic signaling means that there is non-specific activation of multiple functional units, and undesirable behaviors are no longer suppressed. (iii) Dopamine antagonists enhances the activity of indirect pathway neurons, but without regional specificity, meaning that desirable behaviors are also suppressed

Ach – acetylcholine, DA – Dopamine, D2R – dopamine D2 receptor, M1R – M1 muscarinic receptor, MSN – medium spiny neuron

Striatal Function

New findings regarding striatal anatomy and neurochemistry have also refined our understanding of the striatum's functional architecture. We now discuss how recent neurobiological advances relate to our understanding of the striatum's role in behavioural selection, salience processing, and habit formation.

The striatum's output pathways include the **direct** and indirect pathways, where D1 expressing MSNs project *directly* to the output nuclei of the basal ganglia, and D2 type MSNs project *indirectly* via the pallidum (Figure 3). Recent findings suggest, however, that these pathways are less distinct than previously thought, with D1 and D2 MSNs of the ventral striatum often not adhering to the direct and indirect pathways respectively [29,30]. While these classical pathways better describe the architecture of the dorsal striatum, inter-pathway communication has also been demonstrated here - lateral projections from indirect pathway MSNs inhibit direct pathway MSNs via GABA release, and striatal dopamine release reduces this GABA release thereby supressing this lateral inhibition [31]. In the opposite direction, direct pathway neurons show collaterals that bridge across to the indirect pathway [32]. Moreover, recent findings indicate that neural activity in both pathways increases when animals initiate a behaviour, in contrast to the classical accelerator/brake model [33,34]. One interpretation of this finding is that the activation observed in the indirect pathway corresponds to the suppression of alternative, undesirable behaviours [35], and that this suppression may occur via collaterals between direct and indirect pathway MSNs [35](Figure 2B).

The activity of mesostriatal dopamine neurons has been shown to signify the discrepancy between expected rewards and actual rewards, which has been termed the reward prediction error [36]. In addition to reward processing, some models have emphasised mesostriatal dopamine neurons' role in assigning **salience** to environmental stimuli, and its potential relevance to the development of psychotic

symptoms [1]. While it has been debated whether mesostriatal dopaminergic neurons carry information regarding salience that goes beyond reward related information [37,38], recent studies suggest that while dopamine signalling within the ventral striatum is strongly linked to stimulus value, dopamine signalling in more dorsal regions does not track value, but rather the novelty and intensity of stimuli, and in particular *threat* related information [13,39,40]. It has also been recently demonstrated that optogenetic stimulation of dopamine neurons is sufficient to imbue unremarkable environmental stimuli with motivational properties, and that these stimuli are subsequently able to evoke dopaminergic activity, despite never having possessed any intrinsic salience [41]. Recent human PET-fMRI studies also provide evidence that striatal dopamine has a broader role than simply encoding reward prediction errors, and is associated with the function of cortical salience networks [42], and making more general inferences about the state of the environment [43].

Striatal function along a ventral-dorsal axis is also relevant to habit formation. During the learning of action-outcome pairings, performance is goal directed and sensitive to changes in outcome values. After extensive training, however, performance moves to a stimulus-response mode that is inflexible, and no longer responds to changes in outcome. This evolution of behaviour from contingency dependent learning to habitual responding has been associated with a shift from ventral to dorsal striatal processing [44]. Supporting this, lesions to nigrostriatal pathways and dorsal striatum disrupt habit formation, **amphetamine sensitisation** encourages habit formation, and it is the dorsal striatum that is implicated in the habitual responses to drug cues experienced by addicts [45]. Recent work has focused more specifically upon the role of the striatal tail, and is consistent with these earlier findings in that this region appears to be involved in storing stable values while the ventral region acts as a flexible coder of information [46].

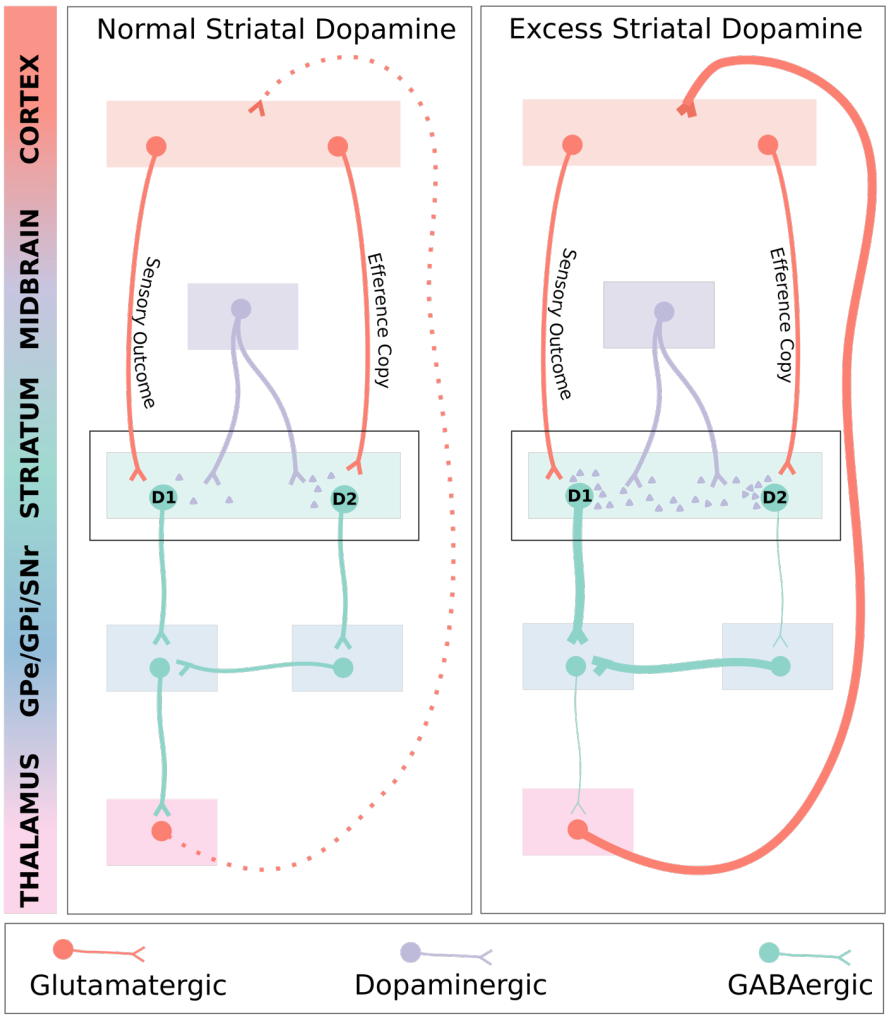

Figure 3. Mechanisms via which excess striatal dopamine may impair efference copy transmission With normal striatal dopamine signaling (left), neurons carrying the efference copy signal preferentially synapse onto D2 expressing GABAergic medium spiny neurons of the indirect pathway. Increased dopamine release within the striatum (right) inhibits these D2 expressing neurons. Excess striatal dopamine may therefore interfere with appropriate transmission of the efference copy signal. In the auditory areas of the dorsal striatum this could result in inner speech being mischaracterized as externally generated

Striatal Dopamine and Schizophrenia

The Mesolimbic Dogma

Early models of schizophrenia proposed that dysfunction of the mesolimbic pathway underlay **positive psychotic symptoms** (Box 1)[4]. Related to that, it was also proposed that newer antipsychotics benefit from mesolimbic selectivity when compared to older agents [47]. While questions were raised regarding the precise locus of striatal dysfunction [48], a focus on mesolimbic pathways persisted, likely due to the absence of robust evidence to refute it. As a result, the mesolimbic hypothesis became a central dogma of schizophrenia, featured in many textbooks, and frequently invoked in discussions regarding the pathophysiology of the illness [1,2,49,50]. As it was not possible to measure limbic dopamine function in vivo, the theory was based on indirect evidence. Moreover, it originated when the dorsal striatum was thought to be solely involved in motor function, and unlikely to be involved in psychosis. Subsequent advances suggest it may in fact be these dorsal regions that play a central role in the pathophysiology of the disorder.

Post-mortem studies of striatal dopamine function

Early post-mortem studies investigating striatal dopaminergic abnormalities produced inconsistent results. Initial studies measured concentrations of dopamine, and while some reported an association between schizophrenia and increased concentrations specifically in the **nucleus accumbens** (NAcc) [51], others found dopamine concentrations elevated specifically in the dorsal striatum [52]. Moreover, tyrosine hydroxylase activity, the rate limiting enzyme in dopamine synthesis, was shown to be elevated throughout the striatum [53], as were dopamine receptor densities[54].

More recently, no differences between patients and controls in terms of either density of dopaminergic terminals [55], or levels of tyrosine hydroxylase have been found in

the NAcc[56,57]. Inferences from post-mortem studies, however, are limited by the fact that most patients have received antipsychotic drug treatment, which may upregulate dopamine receptors [58], and alter presynaptic dopamine function[59],

Clinical Findings

The origins of the mesolimbic hypothesis date back to observations that symptoms, displayed during epileptic seizures localised to limbic areas, were similar to the symptoms of schizophrenia[122]. It was also noted that individuals with tumours in limbic areas were likely to be diagnosed with schizophrenia[123]. Further support came from research using electrodes implanted in individuals with schizophrenia, which demonstrated increased activity in limbic regions during periods of active psychosis[124].

Amphetamine Models

The above mentioned clinical findings were not neurotransmitter specific. The link to dopamine was based on three complementary findings. First, that high doses of amphetamines were able to induce a florid psychotic state [125]; second, that in rodents amphetamine induced dopamine release appeared greatest in the NAcc[126]; and third, that **amphetamine induced stereotypy** was specific to increased dopaminergic transmission in the NAcc [127].

Antipsychotics

Injections of antipsychotics into the NAcc abolished amphetamine induced behaviours, but injections into caudate had no effect [128]. Furthermore, some antipsychotics specifically upregulated dopamine turnover in the NAcc, supporting the hypothesis that dopamine blockade of this region was central to the 'antipsychotic' effect of antipsychotics. This was in keeping with findings that while typical antipsychotic drugs increased c-fos and neurotensin expression in the NAcc and **dorsal striatum**, atypicals affected expression solely in the NAcc, suggesting that the NAcc was central to antipsychotic effects whereas dorsal actions might be solely related to motor side-effects [129].

Together these findings led to the hypothesis that psychosis was due to over-activity in the mesolimbic dopamine pathway [2,49,50]. Modern neurochemical imaging findings, however, suggest that it is within dorsal regions of the striatum that dopaminergic aberrations are greatest[61](Figure I).

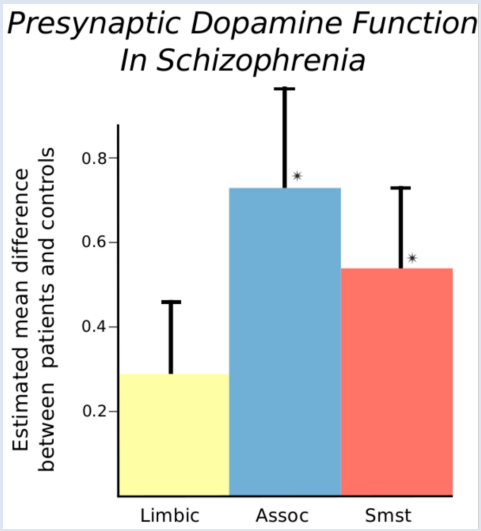

Figure 1: Results of a meta-analysis examining PET measures of presynaptic dopamine in schizophrenia patients and controls[61].
Limbic – Limbic Striatum, Assoc- Associative Striatum, Smst – Sensorimotor Striatum, *- Statistically significant difference between patients and controls ($p<0.05$ in a random effects meta-analysis)

Box 1. The Mesolimbic Hypothesis of Schizophrenia

and furthermore dopamine and its metabolites are greatly affected by death[60].

In vivo neuroimaging of the striatum

Positron emission tomography (PET) allows quantification of the dopamine system in vivo. Radiolabelling of the dopamine precursor L-dihydroxyphenylalanine enables the measurement of its uptake and conversion in dopamine neurons to give an index of dopamine synthesis capacity. Alternatively, imaging the competition between endogenous dopamine and radioligands specific to postsynaptic dopamine receptors can determine baseline levels of dopamine (following depletion of endogenous dopamine using compounds such as a-methylparatyrosine), as well as the magnitude of dopamine release (following a pharmacological or psychological challenge).

Meta-analysis of studies using these PET and SPECT techniques shows that there is a robust increase in striatal dopamine synthesis and release in psychosis [61]. There is no evidence of major alterations in dopamine D2/3 receptors, although it is possible that increased receptor occupancy by raised endogenous dopamine levels masks this, or alternatively that differences between groups in the affinity state of the receptor are not detected with the antagonist ligands generally used [62–64].

Early PET and SPECT studies of presynaptic dopamine function could distinguish anatomical subdivisions of the striatum but lacked sufficient resolution to accurately distinguish between functional subdivisions. Accordingly, these studies reported outcomes for the whole striatum or anatomical subdivisions. However, the subsequent development of higher resolution PET scanners has enabled the assessment of striatal functional subdivisions as well. The first studies to report on these subdivisions in patients with schizophrenia found that the greatest differences in dopamine function were within the associative striatum, with differences in the limbic subdivision not reaching statistical significance [63,65]. The finding that dopaminergic dysfunction is greatest in the associative region has been replicated in

multiple studies since then [63,66–68]. Meta-analysis of these studies found that dopaminergic function was significantly elevated in patients relative to controls in associative (Hedges' g=0.73) and sensorimotor regions (g=0.54), but was not significantly altered in the limbic subdivision (Box 1) [61].

Studies investigating presynaptic dopamine function in individuals at **clinical high risk** (CHR) of psychosis also found the greatest abnormality to be in the associative striatum [65,69], and that conversion from CHR to psychosis is associated with a progressive increase in dopamine synthesis capacity in the dorsal (predominantly sensorimotor) striatum, while no significant change was observed in the limbic subdivision [70].

In a multi-modal study, frontal activation during a working memory task was shown to correlate inversely with associative striatum dopamine synthesis capacity in a CHR group [71]. In the same sample, dopamine synthesis capacity in the associative striatum correlated with greater activation in the left inferior frontal region during a verbal fluency task in the CHR group, but not in the control group [72]. In both studies, no correlations were seen for limbic or sensorimotor subdivisions. fMRI only studies have shown hypoconnectivity between cortex and dorsal striatum in individuals with schizophrenia [73–76], CHR individuals [77], and individuals at genetic risk [73]. Greater activity within the dorsal striatum as measured during resting state MRI has also been shown to correlate with psychotic symptoms [78], and treatment response correlates with increased functional connectivity between associative striatum and prefrontal cortex [79]. Meanwhile, diffusion tensor imaging has found reduced anatomical connectivity between the dorsolateral prefrontal cortex and the associative striatum in schizophrenia [80]. It is important to recognize, however, that some fMRI studies have also shown alterations in ventral striatal function in patients with psychotic disorders relative to controls [81].

In summary, in contrast to the mesolimbic theory, in vivo neuroimaging studies have provided evidence that dopaminergic dysfunction in schizophrenia is greatest within dorsal, as opposed to ventral regions of the striatum.

How could striatal dysfunction lead to the symptoms of schizophrenia?

An ongoing question is how the neurobiological abnormalities identified in patients translate to the diverse psychopathology they present with. We now suggest several mechanisms whereby striatal dysfunction could contribute to the clinical manifestations of the disorder. It is important to note, however, that many of these proposals are speculative at this stage, and furthermore that schizophrenia is a heterogeneous disorder and no single brain region or neurotransmitter is likely to be able to account for all symptoms in all patients.

Aberrant Salience and Delusional Form

Theoretical models linking biological substrates to phenomenological experience in psychosis have frequently built upon the finding that mesostriatal dopamine signalling is involved in marking the salience of environmental stimuli[1]. Excessive spontaneous dopamine transients are proposed to lend irrelevant external or internal stimuli significance due to the temporal association of the stimuli with striatal signalling[1,82]. Although interpretations have often focused upon the mesolimbic pathway, it is apparent that nigrostriatal pathways are also involved in signalling salience[13,39,40,83], and the finding that dopaminergic activity within dorsal regions of the striatum is tied to signalling threat related information[39], may have relevance to the fact that delusions are frequently persecutory in nature.

Existing models have proposed that delusions then develop secondary to cognitive processes attempting to construct a coherent explanation for these unusual

experiences. Historically, however, discussions regarding the phenomenology of delusions have emphasised *form* of thought over *content*. Delusional form is characterised by a certainty of conviction, that is accompanied by an inability to shift perspective, and an imperviousness to counterargument[84]. Given the role of the dorsal striatum in habit formation and the coding of stable values[44,46], one can speculate that the dopaminergic dysfunction of the dorsal striatum that accompanies the onset of psychosis could lead to a more habit oriented mode of cognition, contributing to the rigid *form* of thought as well as its unusual content[70].

Another finding of relevance to theories of aberrant salience attribution in schizophrenia is the following one. A stimulus, even if initially lacking inherent salience, once paired with dopaminergic activity, maintains the ability to evoke dopaminergic activity over time [41]. This suggests that in psychosis, once an environmental stimulus has been 'highlighted' by aberrant dopamine signalling, it may maintain its ability to trigger dopaminergic activity, potentially cementing its position in a delusional framework, even if the system subsequently returns to normal function.

The Dorsal Striatum as an Integrative Hub

The striatum, and specifically areas of the associative striatum, can be viewed as an integrative hub. Regions of the caudate receive inputs from nearly the entire cortex[11,17]; and, additionally, via various connections with the midbrain[13], the associative striatum acts as a moderator of communication between limbic and motor regions.

Given that the striatum performs an integrative role, functional disruption secondary to aberrant dopamine signalling may lead to the associative impairments observed in schizophrenia. Based on preclinical models, it has been proposed that the underlying pathophysiology in schizophrenia represents a combination of increased

aberrant spontaneous phasic dopamine release, and a reduction in adaptive phasic release in response to relevant stimuli[82]. This would lead to increased 'noise' in dopamine signalling in the associative striatum, which could explain findings of reduced functional connectivity between associative striatum and cortex[73], and could disrupt integration of cortical inputs from emotional, cognitive and motor areas. This provides a potential neurobiological correlate for Bleuler's original description of the syndrome as principally resulting from a loss of association between thought processes, emotion and behaviour[85], and could underlie symptoms such as **inappropriate affect**. However, this has yet to be definitively tested.

Regionally targeted dopamine signalling enables the precise selection of specific behaviours over others, and collaterals between direct and indirect pathway MSNs [35] mediate the appropriate integration of multiple signals (Figure 2B). Undirected dopamine transmission will impair this mechanism leading to disorganised behaviour. In contrast, in the context of pharmacological approaches, D2 antagonism will have the ability to supress overactivity within these systems, but will potentially impair the execution of desired behaviours (Figure 2B).

Abnormal Perceptions and Efference Copies

An **efference copy** is an internally generated replica of an outgoing motor signal, that has the effect of dampening sensory perceptions occurring as a result of the motor act, encouraging it to be perceived as self-authored and avoiding attribution to an external agent. The possibility, therefore, that disruption of efference copy mechanisms could contribute to **passivity phenomena** has long been suggested[86].

Efference copies accompanying internally generated motor cortex activity travel via pyramidal tract neurons to the dorsal striatum[87,88]. There is some evidence that the glutamatergic corticostriatal neurons thought to encode the efference copy tend to synapse upon the GABAergic D2 striatal MSNs of the indirect pathway(figure

3)[87,89,90]. One might speculate therefore that excessive dopaminergic signalling within the striatum will inhibit D2 expressing neurons, thereby reducing activity of the indirect pathway and potentially impeding the appropriate transmission of the efference copy signal, meaning that internally generated phenomena may not be coded as such. However, this hypothesis remains to be tested, including in humans, and is only one possible mechanism of disrupted efference copy signalling.

In addition to motor passivity phenomena, a similar mechanism may contribute to auditory hallucinations related to inner speech, such as **thought echo**. Inner speech is in certain respects a motor act, in that it is thought to result from motor plans for speech that are subsequently aborted[91]. Recent research suggests that efference copy mechanisms account for the fact that it is typically easily distinguished from external speech[92]. The neurobiological correlate of inner speech includes neural activation in cortical areas involved in the perception of external speech, such as the secondary auditory cortex[93], and these cortical areas project to the dorsal striatum[18]. Abnormalities of the dorsal striatum have been associated with auditory hallucinations in studies of brain structure, metabolic rate and perfusion, and it is possible that the mechanism discussed above may contribute to the relationship between these symptoms and striatal dysfunction[94–96].

Efference-copy mechanisms, however, are less likely to account for auditory hallucinations that are phenomenologically unrelated to inner speech, and a predictive coding framework, which is a more generalizable model, seems more relevant in this context. Predictive coding refers to the idea that the brain compares prior expectations with new sensory evidence, and uses the discrepancy between the two (the prediction error) to update its model of the world. The certainty regarding one's prior expectation is described as the 'precision' of that prediction. Both the extent of the difference between prior and sensory data, and the precision of these determine the magnitude of the prediction error[97]. Recent research has

demonstrated that auditory hallucinations are related to a greater ability of priors to influence perception[98]. It was also demonstrated that amphetamine induced dopamine release in the associative striatum is associated with this increased weighting of priors[99]. These findings are complemented by recent preclinical research showing that neurons in the tail of the striatum code prior beliefs regarding the value of auditory stimuli[100].

Cognitive and Negative Symptoms

It addition to the role that striatal dopamine signalling plays in the development of positive symptoms, several mechanisms have been suggested for its contribution to the cognitive and negative symptoms of the disorder as well.

Cognitive impairments in schizophrenia have been suggested to result from cortical hypodomaminergia, an idea supported by the importance of cortical dopamine signalling for prefrontal related cognition [101,102]. Recent work in rodents has extended this framework to include striatal involvement, by showing that the relationship between cortical and striatal dopamine is bidirectional, and that increased dorsal striatal dopaminergic signalling can reduce mesocortical dopamine release and produce cognitive deficits[103,104]. While in vivo imaging evidence for a deficit in cortical dopamine transmission has emerged[105], and the multimodal studies discussed above have suggested that striatal dysfunction may be functionally linked to cortical hypofunction, the direction of causality remains unclear and has yet to be determined, including in human studies[71,72].

Striatal hyperdopaminergia could conceivably result in cognitive impairments either by disrupting signalling between frontal cortex and associative striatum, or by potentially driving cortical dopamine dysregulation[103]. Of relevance to this question is an animal model of striatal D2 receptor overexpression, designed to mimic the increased striatal dopamine signalling observed in schizophrenia [103,106].

Studies using this model found that striatal D2 overexpression led to both reductions in cortical dopamine turnover and cognitive deficits[106]. These abnormalities persisted even following the normalisation of striatal dopamine transmission, suggesting that whilst increased striatal dopamine signalling may lead to cognitive impairments, subsequent adaptive changes may underlie their persistence[106]. This could contribute to the finding that the attenuation of striatal dopamine transmission with antipsychotics is of limited benefit in treating cognitive symptoms. This potential role of associative striatum, is also supported by in vivo studies showing that reduced connectivity of the associative striatum and substantia nigra is related to the severity of cognitive deficits in schizophrenia[75,107].

There is also evidence that striatal dysfunction may contribute directly to negative symptoms. Studies employing probabilistic learning tasks have shown that negative symptoms in individuals with schizophrenia may be related to impaired reward-based learning [108–111]. Multiple studies have demonstrated that the striatum plays a key role in the orchestration of this type of behaviour[112], and it has been proposed that excessive *aberrant* dopamine release may mask *adaptive* striatal dopamine release, thereby contributing to these behavioural deficits in schizophrenia[111]. This is consistent with neuroimaging studies showing reduced midbrain and striatal activation during reward processing in patients[81,113].

Therapeutic implications

In this section we consider the implications of the evidence discussed earlier for the development of new treatments for schizophrenia that are not D2 receptor blockers and that might address the striatal dopamine dysfunction seen in the disorder.

As previously discussed, studies have shown that the magnitude of dopaminergic abnormalities in schizophrenia varies across the striatum, with the most marked dysfunction seen in the associative striatum. These findings suggest that anatomically selective modulation of dopamine function would be preferable[61]. This could have the potential benefit of reducing adverse effects that may occur secondary to dopamine antagonism in regions such as the cortex, where PET imaging evidence indicates dopamine signalling may be unaltered or even reduced in schizophrenia.

The existing mechanistic understanding of striatal circuitry points at some possible therapeutic approaches that could allow more anatomically precise modulation of striatal dopamine function. One such mechanism, as discussed earlier, is muscarinic modulation of striatal dopamine release. Preclinical studies have shown that M4 a positive allosteric modulators (PAM) act on striatal MSNs to specifically inhibit *dorsal* striatum dopamine release via endocannabinoid signaling [114,115]. Other preclinical studies have shown that activation of group 1 metabotropic glutamate receptors may also selectively reduce dorsal striatum dopamine transmission via interaction with M4 receptors, but that unlike M4 activation, mGlu1 PAMs appear to have the advantage of not reducing motivational responding[115]. Encouragingly, a PAM of the M1/M4 receptor has shown efficacy in treating schizophrenia, although tolerability issues have prevented further clinical trials as of yet[116].

Concluding remarks and future perspectives

Recent in vivo imaging evidence consistently suggests that the major abnormality in dopamine function in schizophrenia is located within the dorsal rather than the limbic striatum. Increasing knowledge regarding the structure, function, and neurochemistry of the striatum has improved our understanding of how these dopaminergic abnormalities may lead to symptoms. While these developments highlight potential pathways for the development of new treatments, the translation of these advances to meaningful clinical interventions, remains a significant challenge (See Outstanding Questions).

Outstanding Questions

Is anatomically precise modulation of dopamine signalling within the striatum possible? The variation across the striatum both in terms of dopamine receptor distributions, and of the mechanisms that control striatal dopamine and striatal function, suggests that it may be, but this remains to be tested.

Do endocannabinoids, GABAgeric, cholinergic, and glutamatergic interventions have therapeutic potential given their role in modulating striatal function?

Do primary striatal abnormalities exist in schizophrenia, or is dysfunction entirely secondary to upstream pathology? Genes associated with schizophrenia, overlap significantly with genes expressed by MSNs but not with those expressed by dopamine neurons. Abnormalities such as patch-matrix differentiation, or ones associated with cholinergic interneurons might also contribute to striatal dysfunction in schizophrenia. Recently, greater densities of afferent excitatory synapses have been found in the NAcc of individuals with schizophrenia, this could, via the feedforward mechanisms discussed (figure 1A), drive increased dopamine release in the dorsal striatum.

Are specific symptoms associated with dopamine dysregulation in specific striatal loci? For instance, are verbal hallucinations associated with dopaminergic abnormalities in striatal regions displaying connectivity to the secondary auditory cortex? Are motor symptoms associated with the motor striatum?

What is the optimal striatal parcellation? Preclinical findings suggest current atlases may oversimplify the picture, and point at the importance of improving our understanding of striatal connectivity. Could we improve our ability to characterise striatal dopamine dysfunction by using individual participant functional or anatomical connectivity to parcellate the striatum in multimodal studies of schizophrenia?

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3. Defining the Locus of Dopaminergic Dysfunction in Schizophrenia: A Meta-analysis and Test of the Mesolimbic Hypothesis

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Abstract

Background

Studies using positron emission tomography to image striatal dopamine function, have demonstrated that individuals with schizophrenia display increases in presynaptic function. Mesolimbic dysfunction specifically, has previously been suggested to underlie psychotic symptoms. This has not been directly tested in vivo, and the precise anatomical locus of dopamine dysfunction within the striatum remains unclear. The current paper investigates the magnitude of dopaminergic abnormalities in individuals with schizophrenia, and determines how the magnitude of abnormality varies across functional subdivisions of the striatum.

Methods

EMBASE, PsychINFO and MEDLINE were searched from January 1, 1960, to December 1, 2016. Inclusion criteria were molecular imaging studies that had measured presynaptic striatal dopamine functioning. Effects sizes for whole striatum and functional subdivisions were calculated separately. The magnitude of difference between functional subdivisions in patients and controls was meta-analysed.

Results

21 eligible studies were identified, including 269 patients and 313 controls. Individuals with schizophrenia (Hedges' $g=0.68$, $p<0.001$) demonstrated elevated presynaptic dopamine functioning compared to controls. Seven studies examined functional subdivisions. These demonstrated significant increases in patients compared to controls in associative ($g=0.73$, $p=0.002$) and sensorimotor ($g= 0.54$, $p=0.005$) regions, but not limbic ($g=0.29$, $p=0.09$). The magnitude of the difference between associative and limbic subdivisions was significantly greater in patients compared to controls ($g=0.39$, $p=0.003$).

Conclusion

In individuals with schizophrenia dopaminergic dysfunction is greater in dorsal compared to limbic subdivisions of the striatum. This is inconsistent with the mesolimbic hypothesis and identifies the dorsal striatum as a target for novel treatment development.

Keywords: PET, neuroimaging, nigrostriatal, F-DOPA, amphetamine

Introduction

Dysfunction of the dopamine system is one of the most well established findings in schizophrenia.[1–4] Initial evidence was mostly indirect: based on preclinical work, the behavioral effects of drugs, and post-mortem studies.[5] The development of positron emission tomography (PET) and single-photon emission computed tomography (SPECT), allowed the dopamine system to be studied *in vivo* in individuals with schizophrenia.[6] Initial studies employed ligands specific to dopamine receptors, and allowed the quantification of receptor availability, while later work was able to investigate dopamine synthesis and release, and other aspects of dopaminergic function. Previous meta-analyses of these imaging studies have found that the major dopaminergic abnormality in schizophrenia is increased presynaptic activity in the striatum.[1,3] While an elevation of postsynaptic D2 receptors has also been proposed, meta-analytic findings have been less convincing,[1] although the presynaptic results raise the possibility that receptor differences may be masked by increased endogenous dopamine levels.[7–9]

Although cortical dopaminergic functioning has also been studied in schizophrenia,[10,11] the main anatomical focus for investigations of dopamine dysfunction has been the striatum. Animal research has demonstrated that the striatum can be divided into three distinct sub-regions based on function and the predominant topography of brain projections from limbic, associative and sensorimotor cortical areas to the striatum (see Figure 1).[12,13] The antero-ventral striatum receives projections from limbic areas such as the orbital frontal cortex and medial temporal lobe, and consequently has been termed the limbic striatum. Anatomically it comprises the nucleus accumbens, and ventral parts of the caudate and putamen. The associative striatum, involved in higher cognitive function, receives projections primarily from cortical regions involved in executive and other higher cognitive processes, such as the dorsolateral prefrontal cortex, and is made up

of the majority of the caudate, and the precommisural putamen. Finally, the sensorimotor striatum, involved in sensory and motor processing, receives afferent projections predominantly from sensory, motor and premotor areas and consists of the postcommisural putamen. More recent imaging studies have indicated that this topography is paralleled in the human brain.[14,15]

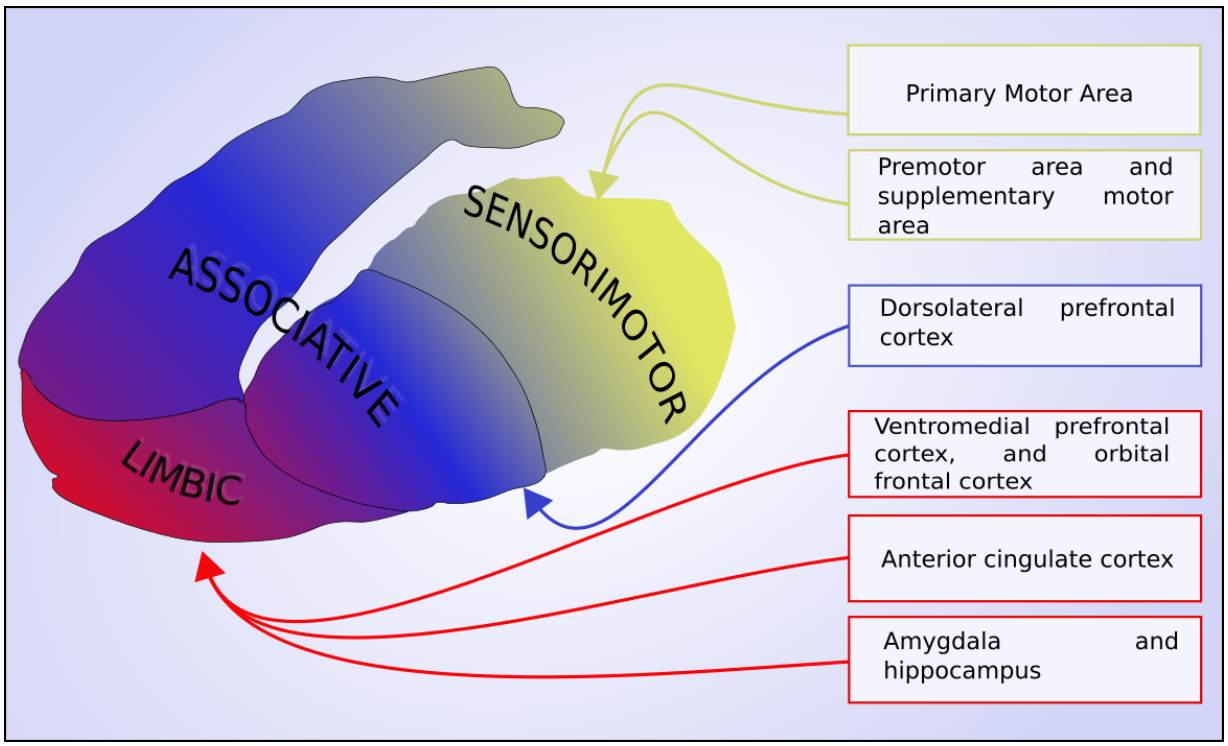


Figure 1: The topography of cortical afferents to the striatum illustrating the functional subdivisions.
Red indicates the limbic striatum, blue the associative striatum and yellow the sensorimotor striatum.

Primarily based on preclinical research, dopaminergic hyperactivity of the limbic striatum has long been hypothesised as underlying psychotic symptoms.[16–18] In vivo evidence for a specific mesolimbic abnormality has, however, been lacking. Initial imaging studies did not have sufficient resolution to visualize these subdivisions, and so reported values for either the whole striatum, or the anatomical divisions of caudate and putamen. However, improvements in PET cameras over the past decade have subsequently allowed dopaminergic function to be measured in these functional subdivisions. Work undertaken by Laruelle, Mawlawi, Martinez and colleagues,[19,20]

defined these subregions based on anatomical landmarks to allow the consistent reporting of subdivision findings in PET studies, and Howes, Egerton and colleagues determined the reliability of this approach.[21] Initial studies using these functional divisions suggested that the greatest abnormality was within the associative striatum.[8,22] Several further studies have since been performed, but the results have not been meta-analysed.

In the current paper, we aim to test the mesolimbic hypothesis by comparing the magnitude of dysfunction between the limbic and other striatal subdivisions. We also provide an update to previous meta-analyses of striatal dopamine function given that a significant number of studies have been published since previous reviews.

Methods

EMBASE, PsychINFO and MEDLINE were searched from 1960 (or 1974 in the case of EMBASE), to December 31, 2016. Titles and abstracts were searched for the words:_("schizophrenia" or "psychosis" or "schizophreniform") AND ("Positron Emission Tomography" or "PET" or "Single photon emission tomography" or SPET or "Single Photon Emission Computed Tomography" or SPECT) AND (Dopamine).

For the meta-analysis of presynaptic dopamine function in schizophrenia the inclusion criteria were: 1) studies of patients with schizophrenia diagnosed in accordance with criteria specified in the Diagnostic and Statistical Manual for Mental Disorders (DSM), or the International Classification of Diseases (ICD)[23,24] and a control group; 2) reporting molecular imaging measures of presynaptic dopaminergic function (see supplementary methods for further details) for both the patient and control groups; 3) providing data enabling the estimation of mean difference between control and clinical groups for the dopaminergic measure; 4) for the subdivision analysis only studies reporting all three subdivisions (limbic, associative and sensorimotor subdivisions) were included to enable comparisons across regions.

Studies reporting data on dopaminergic functioning in individuals with treatment resistant schizophrenia, or co-morbid substance dependence, were excluded. This is because the primary neurobiological abnormality in these patients may not involve striatal hyperdopaminergia.[25–28]

Data extraction

The primary outcome of interest was the dopamine imaging parameter reported for the patient and control groups. For studies using labelled L-DOPA this was the influx constant in the region of interest relative to uptake in the reference region, while for studies using a release or depletion paradigm this was percent change in

binding potential. In addition, author, year of study, number of participants, participant age and gender, illness duration, antipsychotic treatment, symptom scores, scan length, and whether an arterial input function was used were extracted.

Two studies[22,29] reporting data in individuals with schizophrenia were not included due to sample overlap with Howes et al 2013.[30] Where values for the whole striatum were not given but data for the caudate and putamen were reported, whole striatum values were calculated as described previously[1] by weighting these values by their volumes as reported in the Oxford-GSK-Imanova Structural–Anatomical Striatal Atlas (43% and 57% respectively). If the ventral striatum was also reported the following weightings were used to derive a summary outcome for the whole striatum: caudate – 36%, putamen – 48%, ventral striatum – 16% [31].

Statistical analysis

All statistical analyses were carried out using the 'metafor' package (version 1.9-9) in the statistical programming language R (version 3.3.1). A minimum of three studies was required for meta-analysis. Standard effect sizes (Hedges' g) for individual studies were estimated. The individual study effect sizes were then entered into a random effects meta-analytic model using restricted maximum likelihood estimation. I^2 values were calculated to estimate between study heterogeneity. Where there were at least ten studies included in a meta-analysis, funnel plots were constructed and visually inspected, and Egger's regression test performed to check for the possibility of publication bias.[32] Secondary subgroup and meta-regression analyses were undertaken to investigate the relationship between dopaminergic function and antipsychotic treatment (studies where ≥75% of patients were antipsychotic naïve were grouped as studies of predominantly antipsychotic naïve patients), scan length, paradigm type, modelling techniques, patient age and severity of symptoms.[33] The statistical significance of differences between subgroups was tested for by fitting separate random effects models for each subgroup, and then

comparing the subgroup estimates in a fixed effects model with a Wald-type test. A significance level of $P<0.05$ (two tailed) was used for all analyses.

To test the hypothesis that dopamine dysfunction is primarily located in limbic regions we first determined if there was a significant difference between patients and controls for each individual subdivision. We next calculated the magnitude of subdivision differences within group, and then determined whether the size of these differences significantly differed between groups (see below and supplementary information for further details).

In order to contrast and quantify the degree of dysfunction between subdivisions, a meta-analysis of difference was undertaken. In this we performed an inter-group (patient vs control) comparison of the magnitude of intra-group subdivision differences (e.g. associative vs limbic). This approach employs methods used to quantify the propagation of errors.[34] For each study, mean within subject differences in presynaptic function between subdivisions were calculated for both patient and control groups. For example, for patients the mean difference between associative and limbic measurements $(\overline{P}_{al})$ equals:

$$\overline{P}_{al} = \overline{P}_a - \overline{P}_l$$
$$(\overline{P}_a = mean\ associaitve\ value)$$
$$(\overline{P}_l = mean\ limbic\ value)$$

In order to calculate the standard deviation of this mean difference, a correlation coefficient for presynaptic functioning between subdivisions is required (see supplementary information and eFigure 1 for full methods).[35] We estimated this correlation coefficient from individual data for 37 subjects (21 controls and 16 individuals with schizophrenia).[36] This showed Pearson's coefficients of 0.72, 0.84, and 0.87 for correlations between sensorimotor-limbic, associative-limbic, and

associative-sensorimotor divisions respectively. To be conservative the lowest of these values (0.72) was used for all comparisons. For example, to calculate the standard deviation of the limbic-associative difference in a patient group[34]:

$$\sigma_{P_{al}} = \sqrt{\sigma_{P_a}^2 + \sigma_{P_l}^2 - 2r_{al}\sigma_{P_a}\sigma_{P_l}}$$

Pal = Standard deviation of limbic-associative difference

Pa = Standard deviation of associative subdivision values

Pl = Standard deviation of limbic subdivision values

r_{al} = correlation between limbic and associative subdivision values

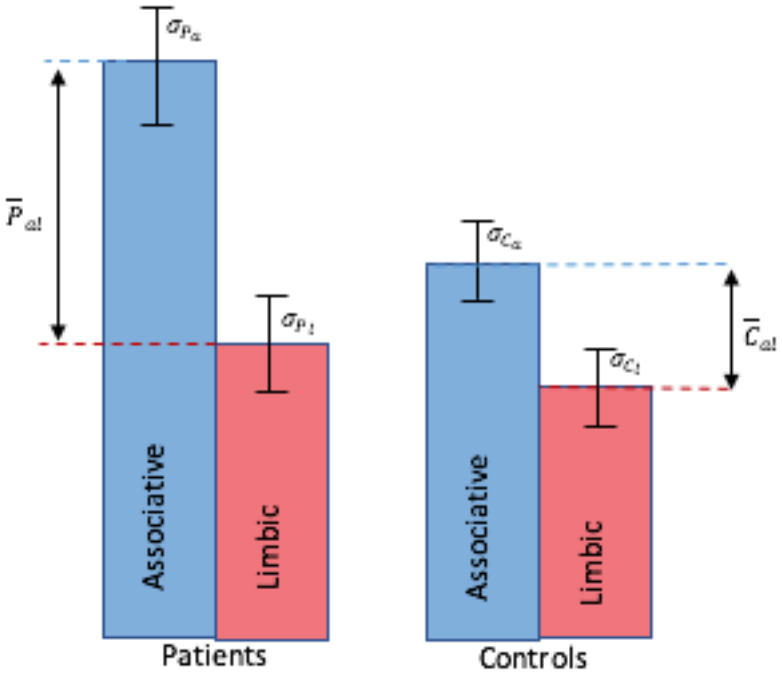
We repeated the exercise to calculate the control mean difference ($\overline{C}_{al}$), and standard deviation ($\sigma_{C_{al}}$), and then calculated the combined standard deviation of both groups ($\sigma_{PC_{al}}$).

$$\sigma_{PC_{al}} = \sqrt{(\sigma_{P_{al}}^2 + \sigma_{C_{al}}^2)/2}$$

The between groups effect size for the study was then calculated for each sub-division using this standard deviation as follows:

$$ES = \frac{\overline{P}_{al} - \overline{C}_{al}}{\sigma_{PC_{al}}}$$

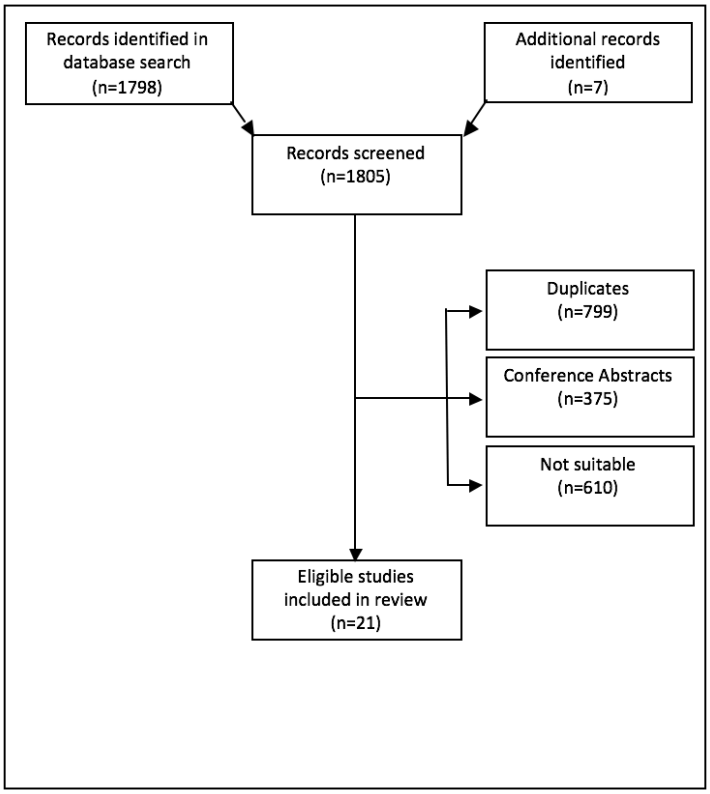
This was converted to the bias corrected Hedges g,[37] which was then entered into the standard meta-analytic model described above. For further information regarding methods see supplementary information.



eFigure 1: Values used in the meta-analysis of difference.
For illustrative purposes only – in reality the limbic values are typically larger than the associative values for controls.

Results

A total of 1798 papers were identified. 21 of these met inclusion criteria (PRISMA flow diagram in eFigure 2 in the supplement).



eFigure 2. Flow diagram illustrating study selection

Studies of the Whole Striatum

21 studies of individuals with schizophrenia met inclusion criteria (see table 1 for study details). The studies included a total of 269 patients (256 with a diagnosis of schizophrenia, 3 schizoaffective disorder, and 10 a mixture of schizophrenia/ schizophreniform disorder) and 313 controls. Presynaptic dopamine function was significantly elevated in individuals with schizophrenia relative to controls with a summary effect size of 0.68 (see Figure 2., 95% C.I. 0.44-0.91; P<0.001). Egger's regression test was not significant (z=1.21, P=0.23), indicating publication bias was unlikely. Visual inspection of the funnel plot potentially suggested asymmetry (see eFigure 3), but a trim and fill analysis did not indicate any missing studies. The I^2 value was 42.5%, suggesting a low to moderate level of heterogeneity. Subgroup meta-analysis of studies of predominantly drug naïve patients, and of patients who were receiving antipsychotic treatment, found a greater effect size in drug naïve patients (g=0.78, P<0.001 and 0.64, P<0.001 respectively, see eFigure 4) but this difference was not statistically significant (p=0.59). Studies using a challenge or depletion paradigm (g=0.95, P<0.001) showed a greater effect size when compared to those using labelled L-DOPA (g=0.52, P<0.001), and this difference was statistically significant (P=0.049, see eFigure5). Neither scan time (P=0.44) nor the use of an arterial input function (P=0.55) was significantly associated with magnitude of effect size in the labelled L-DOPA studies. Meta-regressions of effect sizes against age (p=0.29), total symptoms (p=0.16), and positive symptoms (p=0.39) were not significant.

Study	Controls		Patients								Scan details	
	N	Age mean (SD)/yr	N	Age	Diagnosis	Illness Duration/ months	Antipsychotic treatment	Total symptom score	Positive symptom score	Negative symptom score	Outcome measure	PET Tracer and method
Reith 1994[60]	13	36(13)	5	38(4)	Scz	168	4 naïve, 1 free >3yrs	PANSS 58	PANSS 14(3)	PANSS 12(2)	K_3	[^{18}F]DOPA
Hietala 1995[61]	8	27 (7)	7	26 (7)	Scz	24	All naive	PANSS 81(14)	na	na	K_i	[^{18}F]DOPA
Dao-Castellana 1997[62]	7	25 (5)	6	26 (9)	Scz	72	2 naïve 4 free ≥4 months	PANSS 94 (na)	PANSS 21 (12)	PANSS 33 (7)	K_i	[^{18}F]DOPA
Breier 1997[63]	12	29.2 (9.0)	11	32.4 (10.0)	Scz	79.2	4 naïve, 7 free for >14 days	BPRS 28.8 (7.2)	BPRS 6.7 (2.8)	na	% ⊠ BP_{ND}	[^{11}C]Raclopride AMPH challenge
Hietala 1999[64]	13	30.4 (9.4)	10	29.6 (8.8)	7 Scz 3 SczAf	7	All naïve	PANSS 77.6 (na)	Na	na	K_i	[^{18}F]DOPA
Lindström 1999[65]	10	n/a	12	31(na)	Scz	31	12 naïve, 2 drug free > 2yr	na	na	na	K_i	[^{11}C]DOPA
Laruelle[a] 1999[40]	36	40 (9)	34	40 (9)	Scz	na	7 naïve, 27 free mean 104 days	na	17.5 (6.2)	16.8(6.6)	% ⊠ BP_{ND}	[^{123}I]IBZM AMPH challenge
Elkashef 2000[45]	13	34.6 (10.8)	19	36.3 (na)	Scz	207.6	10 medicated 10 drug free	na	na	na	uptake ratio:str/ ref	[^{18}F]DOPA
Abi-Dargham 2000[9]	18	31 (8)	18	31 (8)	Scz	na	8 naïve, 10 free for mean 139 days	66.6	18.2 (6)	13.8(5.4)	% ⊠ BP_{ND}	[^{123}I]IBZM AMPT
MeyerLindenberg 2002[66]	6	34 (na)	6	35 (na)	Scz	na	All free ≥ 6weeks	na	na	Na	K_i	[^{18}F]DOPA
Kumakura 2007[55]	15	37.3 (6.4)	8	37.3 (6.3)	Scz	na	3 naïve, 6 free for ≥6mths	PANSS 80.2 (4.7)	PANSS 15.4 (3.5)	PANSS 23.6 (4.0)	K_{in}^{app}	[^{18}F]DOPA

Nozaki 2009[67]	20	35.1 (9.5)	18	35.6 (7.4)	Scz	26.4	14 naïve, 4 free	PANSS 79.2 (21.4)	PANSS 22.6(7.3)	PANSS 17.1(6.5)	K_i	[^{11}C]DOPA
Kegeles 2010[8]	18	29 (7)	18	29 (8)	Scz	na	6 naïve, 4 free ≥1yr, 8 free for ≥20 days	PANSS 78.6 (20.6)	PANSS 21.7 (7.1)	PANSS 17.1 (5.9)	% ⊠ BP $_{ND}$	[^{11}C]Raclopride AMPT depletion
Shotbolt 2011[68]	20	43 (12)	7	43 (12)	Scz	na	All medicated	PANSS 56.8 (25.4)	PANSS 13.5 (6.7)	PANSS 15 (4.9)	K_i	[^{18}F]DOPA
Pogarell 2012[69]	7	23.6 (2.7)	8	25.4 (5.8)	Scz	24	Free for 1 week	PANSS 76(18)	na	na	% ⊠ BP $_{ND}$	[^{123}I]IBZM AMPH challenge
Mizrahi 2012[52]	12	26.1 (3.83)	10	24.1 (5.0)	Scz/ Sczform	na	All naïve	na	PANSS 19.0 (3.8)	na	% ⊠ BP $_{ND}$	[^{11}C]-(+)-PHNO MIST
Demjaha 2012[25]	12	44.0 (11.9)	12	44.2 (8.9)	Scz	194.4	All medicated	PANSS 50.7 (5.8)	PANSS 11.9 (2.4)	na	K_i	[^{18}F]DOPA
Howes[b] 2013[30]	29	29.3 (7.5)	29	33.7 (10.6)	Scz	na	16 medicated, 8 free, 5 naïve	CASH 77.6 (47.6)	CASH 38.3 (30)	CASH 31.9 (22.9)	K_i	[^{18}F]DOPA
Caravaggio 2015[70]	10	29.1 (8.4)	3	30 (16)	Scz	na	All medicated	na	na	Na	% ⊠ BP $_{ND}$	[^{11}C]-(+)-PHNO AMPT depletion
Kim 2016[46]	12	30.3 (8.4)	12	31.1 (9.8)	Scz	111.3	All medicated	PANSS 50.3 (11.1)	PANSS 10.8 (2.7)	PANSS 13.2 (5.2)	K_i	[^{18}F]DOPA
Jauhar[36] 2017	22	24.5 (4.5)	16	26.3 (4.4)	Scz	24	11 naïve, 3 free	PANSS 72.9 (16.5)	PANSS 17.8(6.3)	PANSS 18.8(4.1)	Ki	[^{18}F]DOPA

Table 1: Studies of presynaptic dopamine function in individuals with schizophrenia

[a] – includes all subjects from Laruelle et al. (1996)[71,72], Abi-Dargham et al. (1998)

[b] – includes the entire sample from McGowan et al (2004) [29]

AMPH: amphetamine, AMPT: alpha-methyl-para-tyrosine, BP: Binding Potential, BPRS: Brief Psychiatric Rating Scale, CASH: Comprehensive Assessment of Symptoms and History, Ki: utilization rate constant of DOPA relative to a reference region, K_{in}^{app}: net blood-brain DOPA clearance, MIST: Montreal Imaging Stress Test, na: not available, PANSS: Positive and Negative Syndrome Scale, Ref: reference region, Scz: schizophrenia, Sczform: Schizophreniform, SczAf: Schizoaffective disorder, Str: striatum

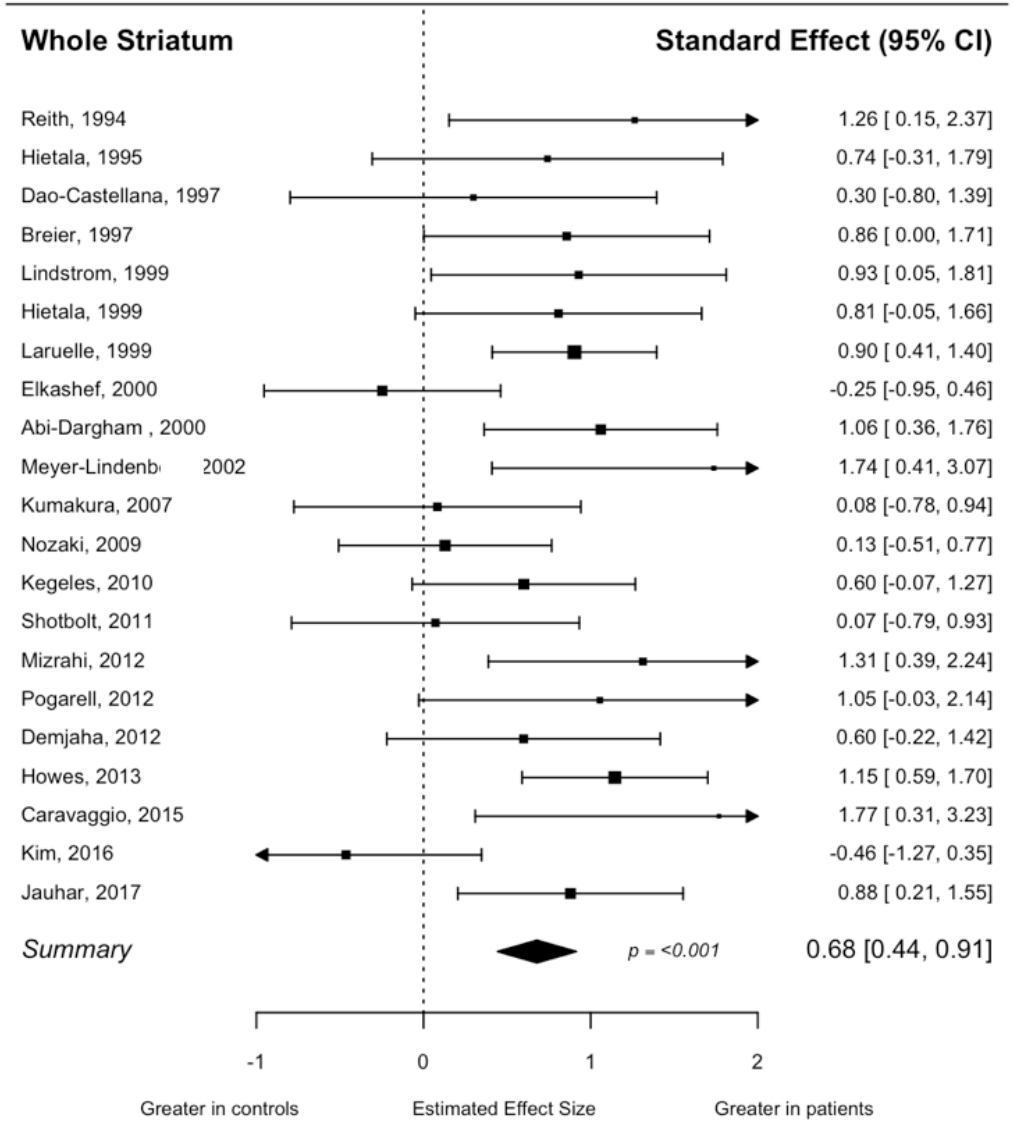
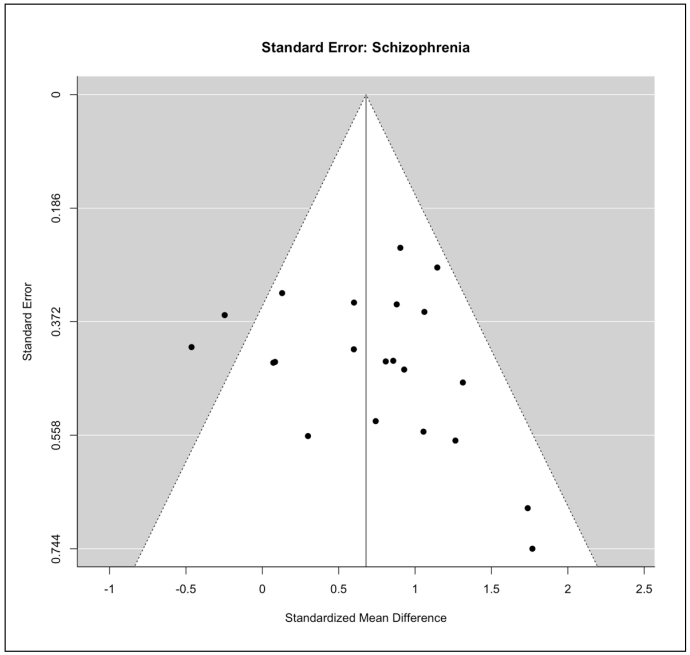
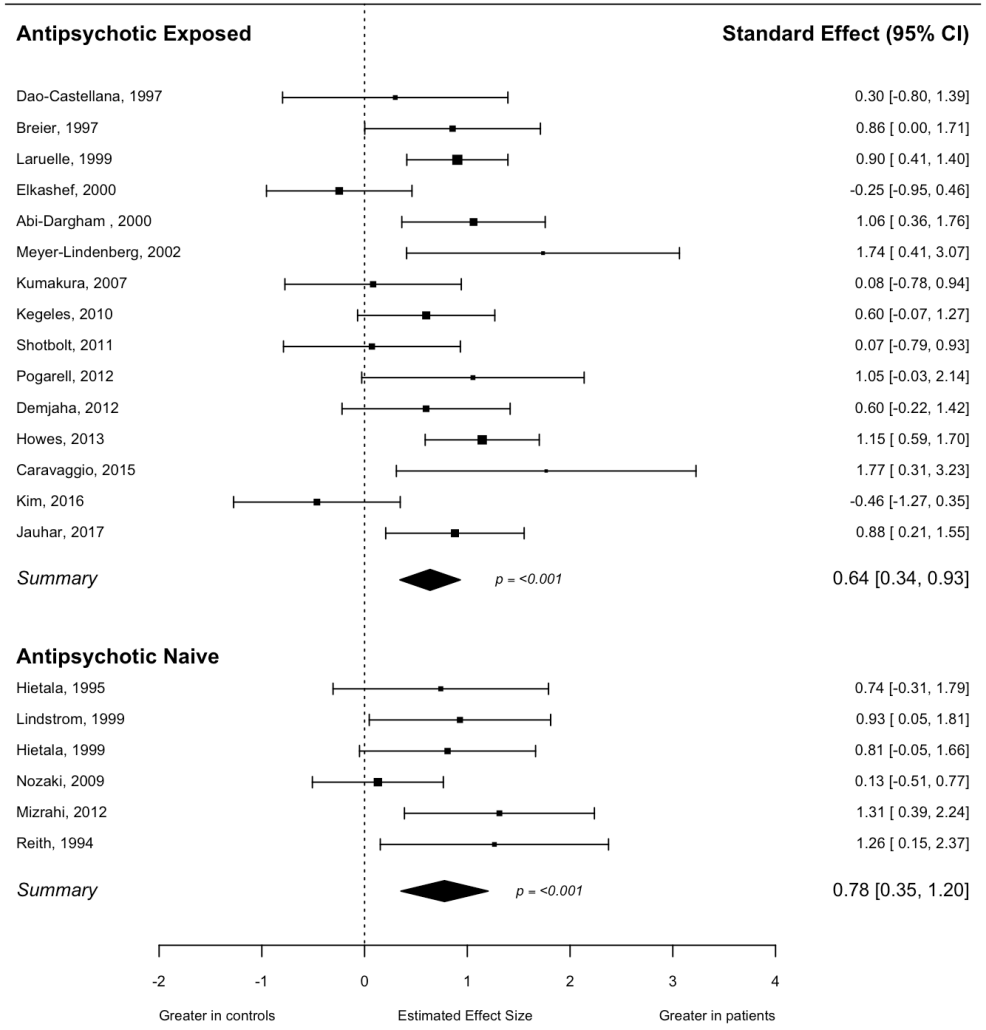


Figure 2. Forest plot of studies investigating presynaptic dopaminergic function in the whole striatum for individuals with schizophrenia.

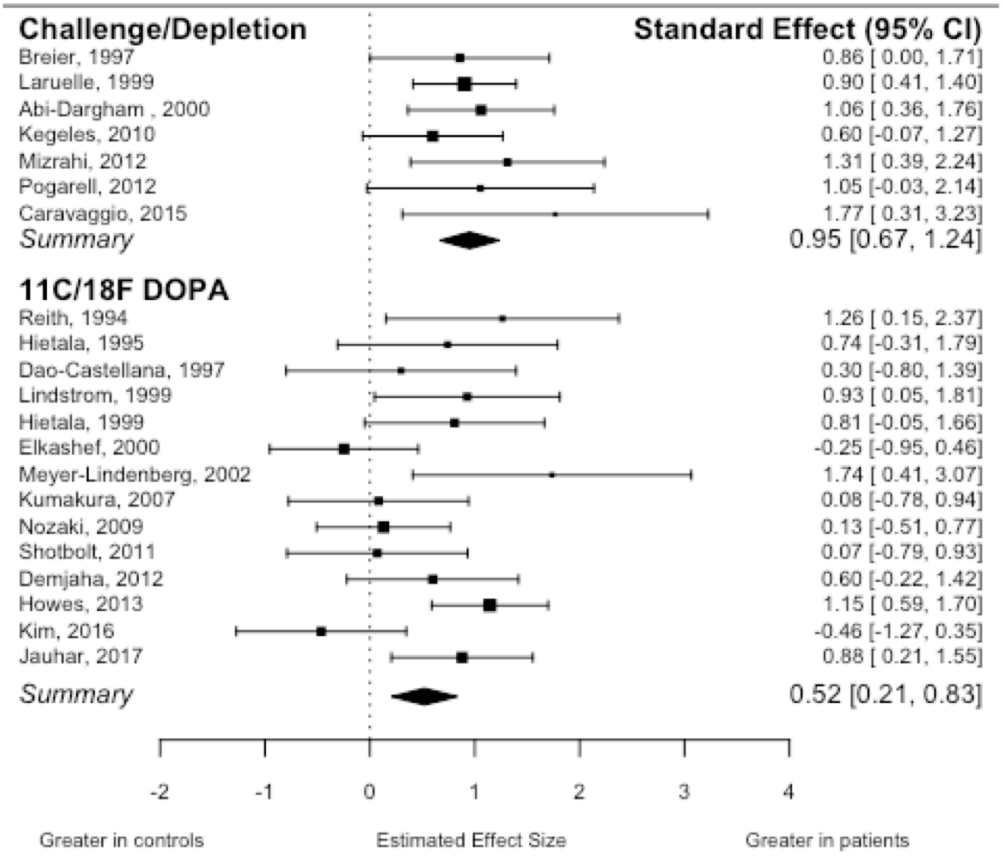
The forest plot shows the effect size (hedges g) and 95% confidence interval for the difference between patients and controls. There is a significant elevation in schizophrenia with a summary effect size of 0.68.



eFigure 3. Funnel plot for studies of presynaptic dopamine function in individuals with schizophrenia.
There is no clear evidence of publication bias.



eFigure 4. Studies of presynaptic dopamine function in individuals with schizophrenia. Studies meta-analyzed separately depending on whether patients antipsychotic exposed or predominantly naïve (≥75% patients naïve).
Significant patient-control differences seen both in individuals who have been exposed to antipsychotics (g=0.65, P<0.001) and antipsychotic naïve individuals (g=0.78, P<0.001).



eFigure 5. Studies of presynaptic dopamine function in individuals with schizophrenia. Studies meta-analysed separately depending on whether a challenge/depletion paradigm or labelled L-DOPA used to index dopamine function.

Significant patient-control differences were seen in both types of study ($p<0.001$ for each), although the summary effect size was significantly greater for the challenge/depletion studies compared to the DOPA studies ($p=0.046$).

Studies of Limbic, Associative and Sensorimotor subdivisions

Seven studies of individuals with schizophrenia reported associative, sensorimotor and limbic subdivisions. These reported data on a total of 104 patients with schizophrenia (schizophrenia or schizophreniform disorder), and 174 controls. All seven studies used the subdivision definitions proposed by Mawlawi et al,[20] and Martinez et al.[19]

Significant differences were found between patients and controls for associative (schizophrenia – g=0.73, p = 0.002) and sensorimotor (schizophrenia – g = 0.54, p=0.009) subdivisions, but not for the limbic subdivision (schizophrenia – g=0.29, p=0.09) (see Figure 3A-C). The results for the associative subdivision showed the greatest heterogeneity (I^2= 58.3%), with sensorimotor (I^2=37.7%), and limbic subdivisions (I^2=29.5%) showing relatively low levels of heterogeneity.

In individuals with schizophrenia, the difference between associative and limbic subdivisions was significantly greater in patients compared to controls (see Figure 3c; effect size, g=0.38, P=0.004). Presynaptic dopaminergic function in schizophrenia was also significantly greater in the sensorimotor compared to the limbic subdivision compared to the difference in controls (g= 0.29, p=0.03). There were no significant patient-control differences as regards the comparisons between dopamine function in the associative and sensorimotor subdivision (g=0.08, p=0.55). These comparisons showed low levels of heterogeneity (associative-limbic I^2= 25.5%, other comparisons I^2=0).

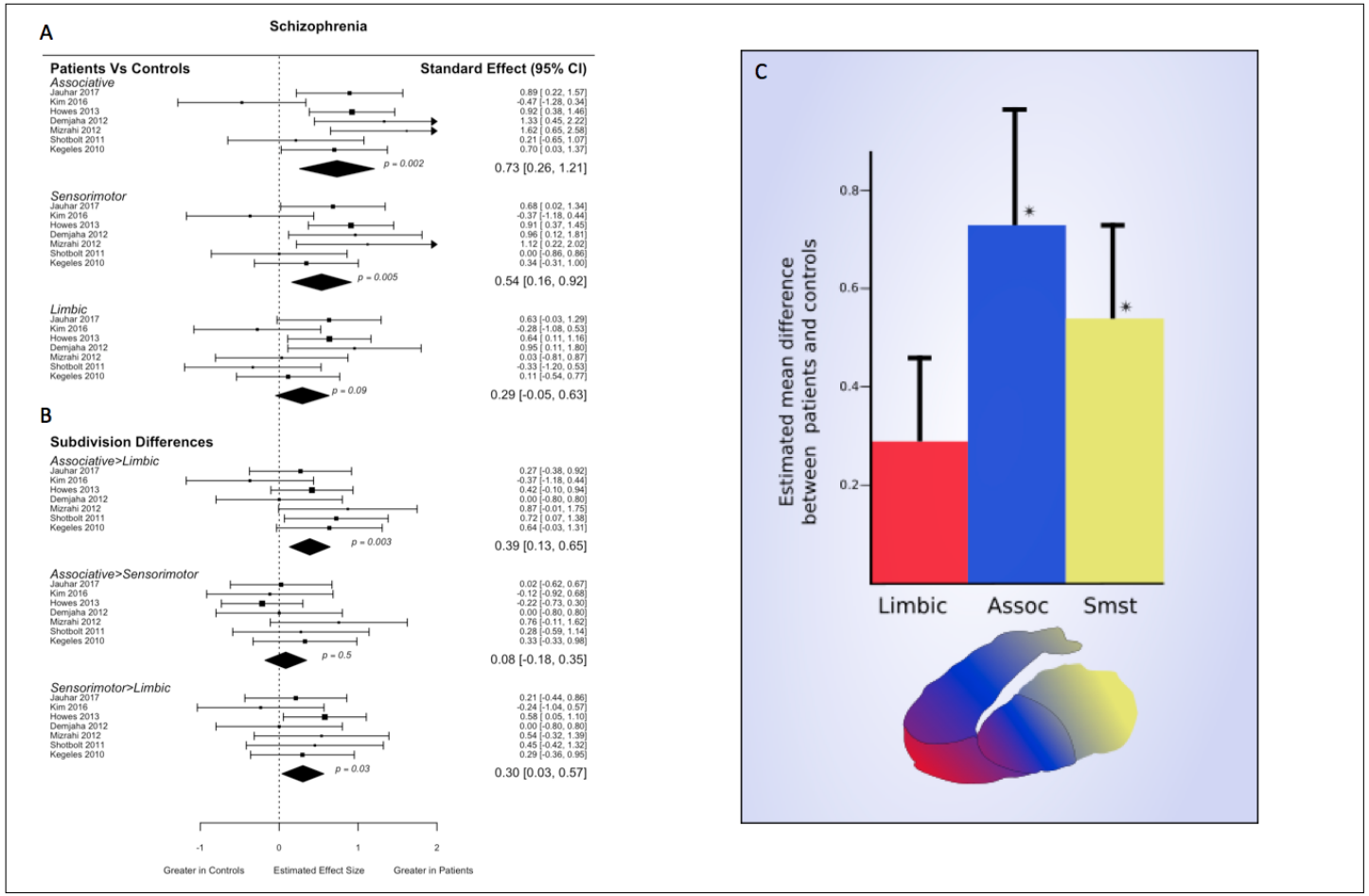


Figure 3. Studies of presynaptic dopamine function in individuals with schizophrenia by functional subdivisions.

Significant elevations are seen for the associative and sensorimotor, but not limbic subdivisions. In schizophrenia the associative-limbic and sensorimotor-limbic differences are significantly greater in patients than in controls

(A) Effect size and 95% CI of difference in dopamine function in schizophrenia between patients and controls showing significant elevations in patients in associative (g=0.73) and sensorimotor (g=0.54) subdivisions but not limbic. (B) Effect sizes and 95% CIs of subdivision differences in schizophrenia between patients and controls. Patients show significantly greater associative-limbic (d=0.38) and sensorimotor-limbic (d=0.29) differences compared to controls. (C) Magnitude of patient-control differences in presynaptic dopamine functioning for striatal subdivisions in individuals with schizophrenia (*p <0.05 for patient-control comparison), error bars represent one standard error).

Discussion

Our main finding is that individuals with schizophrenia display greater elevation in dopaminergic functioning in the dorsal (sensorimotor and associative) relative to limbic striatum compared controls (see figure 3). Moreover, there was no significant difference in presynaptic dopaminergic functioning between patients and controls for the limbic subdivision. This is, to our knowledge, the first study to meta-analyze differences between functional subdivisions of the striatum. Our analysis of the whole striatum included eight additional studies published since previous reviews but is consistent with their findings in showing an increase in schizophrenia.[1,3]

Methodological considerations

Moderate heterogeneity was seen in the studies of individuals with schizophrenia. Methodological factors such as differences in the resolution of scanners, measurement time, experimental paradigm, and modelling technique may contribute to this heterogeneity. In addition, differences in the clinical characteristics of patients could contribute to between study heterogeneity, given findings that increased dopaminergic activity is linked to acute psychosis.[38–40] Some studies included antipsychotic treated patients. However, our sub-analysis in antipsychotic free/naïve patients showed no statistically significant difference between these groups, and the elevation in presynaptic dopamine function was numerically larger in naïve patients than in antipsychotic treated patients, indicating antipsychotic treatment is unlikely to account for the elevation we see. Moreover chronic antipsychotic treatment may reduce dopamine synthesis capacity in some patients.[41]

We combined studies using challenge and depletion paradigms with those using radiolabeled DOPA. Whilst there is some evidence that results from challenge paradigms are directly related to results from radiolabeled DOPA studies,[42,43] it should be recognized that these measures are indexing different, albeit related, aspects of dopaminergic function, and could be influenced by different factors.

Interestingly our sensitivity analysis found that effects were greater for the challenge/depletion studies (efigure 6), which could suggest that these aspects of the dopamine system are particularly affected in schizophrenia.

Another factor contributing to heterogeneity could be the inclusion of individuals with treatment resistant schizophrenia, or with co-morbid substance dependence, given recent findings these groups may show *reduced* presynaptic dopamine functioning.[25,27,28,44] While we excluded studies specifically including these patients, many studies pre-dated these recent findings and did not specify these as exclusion criteria. As such it is likely that some of the included studies may have contained treatment resistant patients; indeed two studies report including patients taking clozapine.[9,45] However this would, if anything reduce effect sizes given treatment resistant patients do not seem to show presynaptic dopamine elevation.[25,46]

We examined the *difference* between subdivisions, as, in the absence of individual patient data, this measure can be more accurately estimated than the *ratio* between subdivisions. A potential drawback of our measure is that if, for example, associative values are greater than limbic values, then a uniform proportionate increase in dopaminergic function across the whole striatum in the clinical group would lead to a greater absolute increase in the associative striatum, and thus give a larger associative-limbic difference. In our case, however, only two of the seven control groups had a value for the associative region that was greater than the limbic value.[30,36] Therefore, if anything, effects related to general increases in striatal functioning would reduce the magnitude of our findings.

When examining the differences between subdivisions, the assumed correlation between subdivisions has an influence on the precision of the estimated magnitude of difference between subdivisions, with a stronger correlation leading to larger effect sizes. The correlation coefficient we employed, however was conservative, using the

lowest of the correlation coefficients between subdivisions that we found in individual participant data. Using the largest coefficient of 0.87 gave an effect size of 0.50 (p=0.01 for associative limbic measure, and 0.29 (p=0.01) for the sensorimotor-limbic measure (See efigure 5). Thus, the differences we report may underestimate the magnitude of the true difference.

The limbic striatum has a smaller volume than either the associative or sensorimotor subdivisions. As a result it is more susceptible to partial volume effects whereby its true activity may be diluted by spill over and spill in from adjacent regions.[47] However, given that there is no consistent evidence of reduced limbic striatal volumes in schizophrenia this would be expected to affect measures in patients and controls equally.[48–51] Moreover one study employed partial volume correction and found a significant elevation in the associative striatum, but not in the limbic striatum in schizophrenia and clinical high risk groups relative to controls,[52] consistent with our meta-analytic findings. The fact that measures of dopamine functioning in the limbic striatum may be less reliable compared to measures in other subdivisions does mean, however, that it is possible the reduced limbic effect size (figure 3A) could be at least partially due to the increased noise inherent in measuring this region.[21,53] This possibility is supported by some[25,52] (but not all[30,36,46]) studies where the variance of the limbic measure, is noticeably greater than the variance of the associative measure.

Neither partial volume effects, nor reduced signal-to-noise, however would account for the patient-control differences found when examining subdivision differences directly (figure 3B). In this case we are, for example, looking at limbic-associative differences in patients, and comparing this to the limbic-associative differences in controls. A reduction in signal-to-noise for the limbic measure will therefore affect patient and control findings equally, and will not bias the results. This means that

while the reduced reliability of limbic measurements may increase the risk of a false negative, in this specific analysis it will not increase the likelihood of a false positive.

The anatomical locus of dopaminergic dysfunction in psychosis

Our meta-analysis confirms, using a larger sample, the previous meta-analytic findings of increased presynaptic dopamine functioning in schizophrenia in the striatum.[1] Moreover, our meta-analysis extends understanding of the nature of dopamine dysfunction in psychosis by showing that the degree of dopaminergic dysfunction varies across the striatum, and identifies the dorsal striatum as the predominant locus of dopamine dysfunction in psychosis. Although patients showed no significant alteration in the limbic striatum relative to controls, we cannot rule out the possibility of a small difference in this subdivision. Nevertheless, in patients the dorsal to ventral balance was significantly shifted dorsally in patients when compared to controls. While a small mesolimbic abnormality may exist, overall these findings are not consistent with a hypothesis which proposes that the predominant locus of dopamine dysfunction is the limbic striatum.

Our findings thus suggest that models highlighting a primary role for excessive mesolimbic dopamine transmission in psychosis may need to be revised.[12–14,28] The associative subdivision receives dopaminergic innervation from the substantia nigra, [12] suggesting that nigrostriatal pathways may be disrupted in schizophrenia. This hypothesis is in keeping with findings of increases in some,[30,55] although not all,[10] aspects of dopamine functioning within the substantia nigra in schizophrenia. The elevation was greatest in the associative striatum, although this was not significantly greater than the elevation in the sensorimotor striatum.

It should be noted, that while our findings support the hypothesis that dopaminergic functioning within the associative striatum may be abnormal in schizophrenia, this does not preclude the possibility that the primary site of dysfunction exists in another

brain region.[5] The associative part of the dorsal striatum receives projections predominantly from dorso-lateral prefrontal cortex.[13] Thus the dorsal locus of dopamine abnormality is consistent with the hypothesis that frontal cortical dysfunction underlies striatal dopamine abnormalities,[16,56] although causality remains to be established in clinical studies.

Our findings also question the proposal that mesolimbic selectivity is a desirable property for pharmacological treatments of schizophrenia,[57] and suggest instead that selectivity for the dorsal, particularly associative, striatum may show advantages in both efficacy and tolerability. Treatment strategies may be able to make use of the neurochemical distinctions found across striatal subdivisions. For example, dopamine transporter densities are greater in the ventral, compared to dorsal, striatum.[58] Due to this variable distribution, combination therapy with a dopamine reuptake inhibitor and D2 antagonist could potentially reduce dopaminergic neurotransmission to a greater degree in the dorsal, as opposed to ventral striatum. There are potential risks to this approach, but evidence suggests that in some patients it may have benefits for the amelioration of negative symptoms.[59]

In conclusion, current molecular neuroimaging studies suggest that in individuals with schizophrenia the major locus of dopamine dysfunction is the dorsal striatum, and significant elevations were not seen in the limbic striatum. These findings are inconsistent with the mesolimbic hypothesis of schizophrenia, and suggest treatments showing nigro-striatal rather mesolimbic selectivity may have better efficacy and tolerability.

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Supplementary Data

The measurement of presynaptic dopamine functioning

Methods for measurement of presynaptic dopamine function included the use of either radiolabelled L-dopa, or a dopamine receptor ligand in combination with a release or depletion paradigm. Radiolabelled L-dopa provides a measure of presynaptic dopamine synthesis capacity.[1,2] Amphetamine stimulates dopamine release from neuron terminals, and inhibits its reuptake to increase extracellular dopamine levels.[3] This can be quantified by measuring the degree to which amphetamine induced dopamine release displaces postsynaptic D2/3 receptor radioligands.[4,5] A similar method can be employed to measure the magnitude of dopamine release in response to a psychological stress.[6] Conversely, the administration of the dopamine depleting agent alpha methyl-paratyrosine, allows intrasynaptic levels of dopamine to be deduced, by measuring the increase in D2/3 radioligand binding following dopamine depletion.[7,8] Together, these paradigms can be taken as measures of presynaptic dopamine functioning.[9]

Calculating whole striatal values from caudate and putamen values

A number of papers did not report whole striatal values but only anatomical subdivisions of caudate, putamen and ventral striatum. In these cases a whole striatal value was calculated based on previously reported methods.[9] Volume based weightings were derived from the Oxford-GSK-Imanova Structural–anatomical Striatal Atlas to give weights of 0.43 and 0.57 respectively.[10] If ventral striatum was also reported we used weights of 0.48, 0.36 and 0.16 for the caudate, putamen and ventral striatum respectively. A correlation of 0.7 for was used (as in the analysis of difference of functional subdivisions). If it was necessary to combine left and right

caudate/putamen, when the bilateral result was not reported, a correlation coefficient of 0.85 was used. Whole striatum standard deviation was calculated as follows:

$$\sigma_s = \sqrt{\omega_p^2\sigma_p^2 + \omega_c^2\sigma_c^2 + 2\omega_p\omega_c r_{pc}\sigma_p\sigma_c}$$

$$\left(\begin{array}{c} \sigma_s = standard\ deviation\ for\ whole\ striatum\ measure \\ \omega_p = weighting\ for\ putamen\ measure \\ \omega_c = weighting\ for\ caudate\ measure \\ \sigma_p = standard\ deviation\ for\ putamen\ measure \\ \sigma_c = standard\ deviation\ for\ caudate\ measure \\ r_{pc} = correlation\ between\ caudate\ and\ putamen\ measure \end{array}\right)$$

Calculating effect sizes for subdivision differences

In order to quantify the difference in dopaminergic alterations between subdivisions a meta-analysis of difference was undertaken. This involved calculating for each study mean within-group differences for subdivisions, and then contrasting patient and control groups against one another. eFigure 1 illustrates the measurements used. The mathematical basis of this comparison is well established and is based upon the concept of propogation of variance.[11] Below follows an illustrative example, comparing associative and limbic subdivisions:

1. We first quantified the within group difference in subdivision means for an individual study:

For patients the mean difference between associative and limbic measurements ($\overline{P}_{al}$):

$$\overline{P}_{al} = \overline{P}_a - \overline{P}_l$$

$$\left(\overline{P}_a = meanassociativevalue\right)$$

$$\left(\overline{P}_l = meanlimbicvalue\right)$$

2. The standard deviation of this difference ($\sigma_{P_{al}}$) can be calculated as follows:

$$\sigma_{P_{al}} = \sqrt{\sigma_{P_a}^2 + \sigma_{P_l}^2 - 2r_{al}\sigma_{P_a}\sigma_{P_l}}$$

This requires the calculation of the correlation coefficient '' between presynaptic functioning in the various subdivisions.[12] Examination of individual patient data from Jauhar et al[13] (in press) showed Pearson's coefficients of 0.72, 0.84, and 0.87 for correlations between sensorimotor-limbic, associative-limbic, and associative-sensorimotor respectively. The lowest (i.e. most conservative) of these values (0.72) was used for all comparisons.

Repeating the exercise for the controls allows the calculation of the control mean difference ($\overline{C}_{al}$), and standard deviation ($\sigma_{C_{al}}$) .

3. The following steps are simply those used to calculate a between groups effect size in the usual manner. In the current study this was performed in R using the *metafor* package. The values calculated above allow for the calculation of the combined standard deviation of both groups:

$$\sigma_{PC\,al} = \sqrt{\frac{(n_P - 1)\sigma_{P_{al}}^2 + (n_C - 1)\sigma_{C_{al}}^2 + n_P\left(\overline{P}_{al} - \overline{PC}_{al}\right) + n_C(C_{al} - \overline{PC}_{al})}{(n_P + n_C - 1)}}$$

Which allows for the calculation of the between groups effect size for the study:

$$ES = \frac{\overline{P}_{al} - \overline{C}_{al}}{\sigma_{PC\,al}}$$

This can be bias corrected in the usual manner to provide Hedges g, which can be then entered into the standard meta-analytic model used previously.

Supplementary References

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4. The Topography of Striatal Dopamine Function and Symptoms in Psychosis: An Integrative PET and MRI study

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Abstract

Striatal dopamine dysfunction has been proposed to underlie symptoms in psychosis, yet it remains unclear how a single neurotransmitter could underlie the diverse and heterogeneous presentations that are observed in clinical practice. One hypothesis is that the symptomatic consequences of aberrant dopamine signalling may vary depending on precisely where within the striatum this dysfunction occurs. We test this hypothesis for the first time using a multimodal imaging approach in 29 unmedicated and minimally-treated patients with first episode psychosis and 21 healthy controls. Patients were clinically assessed using the Positive and Negative Syndrome Scale at baseline, and following a minimum of four weeks' treatment with a dopamine-2 receptor antagonist. Each participant received two brain scans at baseline assessment: one ^{18}F-DOPA positron emission tomography to index striatal dopamine synthesis capacity, and a resting state functional MRI to map corticostriatal functional connectivity. We used these participant-specific functional connectivity maps to derive the preferential cortical connectivity of each voxel within the striatum separately for each participant. Dopamine synthesis capacity within areas of the striatum showing strong functional connections to sensorimotor cortex was related to the baseline motor symptoms ($p=0.01$), and the change in motor symptoms following treatment ($p=0.001$). We also found associations between negative symptoms and striatal dopamine synthesis capacity in striatal regions connected to the default mode cortical network, and between affective symptoms and regions connected to the cinguloopercular cortical network. No relationship was observed between hallucinations and dopamine function in striatal regions connected to auditory regions ($p>0.05$). These findings suggest that the heterogeneity of symptoms and response to treatment observed in psychosis partly results from individual differences in the topography of dopamine dysfunction within the striatum and cortico-striatal circuits.

Keywords: Schizophrenia, Resting State, Bipolar disorder, Connectivity

Introduction

Psychotic symptoms occur across a range of mental illnesses including schizophrenia, bipolar disorder, and psychotic depression. Even when examining a single disorder such as schizophrenia, marked symptomatic diversity exists, with clusters including positive symptoms such as hallucinations and delusions, negative symptoms such as emotional blunting and amotivation, motor symptoms and cognitive deficits (Arndt, 1995; Marder *et al.*, 1997; McGrath *et al.*, 2004; Jauhar *et al.*, 2018a). Given that both symptom and neurobiological abnormalities manifest across multiple mental illnesses (McTeague *et al.*, 2017), there has been an increasing focus on characterising neuronal circuits that have transdiagnostic relevance for understanding psychopathology (Insel *et al.*, 2010; Insel and Cuthbert, 2015). It has been proposed that psychotic symptoms result from aberrant dopamine signalling within the striatum (Abi-Dargham *et al.*, 1998; Laruelle and Abi-Dargham, 1999; Laruelle *et al.*, 1999; Heinz and Schlagenhauf, 2010; McCutcheon *et al.*, 2019), based on findings that dopamine agonists induce psychotic symptoms whilst dopamine antagonists treat them (Bell, 1973; Seeman and Lee, 1975), as well as preclinical studies (Grace, 2016), theoretical models (Maia and Frank, 2017), and *in vivo* measurement of striatal dopamine function using positron emission tomography (PET) (Laruelle *et al.*, 1996, 1999; Abi-Dargham *et al.*, 1998; Heinz and Schlagenhauf, 2010; McCutcheon *et al.*, 2018a). Most work has focused on the link between positive symptoms and dopamine (Laruelle and Abi-Dargham, 1999; Laruelle *et al.*, 1999; Heinz and Schlagenhauf, 2010; Jauhar *et al.*, 2017), and it remains an open question as to whether striatal dopamine alterations are linked to other symptoms seen in psychotic disorders.

The striatum is a central processing hub within the brain, receiving input from almost the entire cortex (Haber, 2016), and as such plays a role in sensory, motor, affective, and cognitive processes (Phillips *et al.*, 2003; Grahn *et al.*, 2008; Guo *et*

al., 2018). Thus, dysfunction in the striatum could disrupt these systems to lead to hallucinations, delusions, emotional blunting and other affective symptoms, motor symptoms and cognitive deficits. Cortical neurons largely project to discrete regions within the striatum, so there is a topographical distribution of cortical inputs to the striatum (Haber, 2016). Dopamine is a neuromodulator that plays a key role in regulating inputs and signal transmission from the striatum. Given the topographical distribution of cortical inputs, this suggests that the localisation of dopamine dysfunction within the striatum may determine the corticostriatal circuits most affected (McCutcheon *et al.*, 2019). For example, dopamine dysfunction within regions of the striatum receiving input from cortical auditory areas might disrupt auditory processing to lead to auditory hallucinations, while dopamine dysfunction within regions connected to motor areas would lead to motor symptoms.

Several recent advances have made it possible to test this hypothesis in vivo. Improvements in the resolution of PET scanners have meant that greater anatomical precision is possible when imaging striatal dopamine. This has led to the finding that dopamine dysfunction in schizophrenia is not uniform across the striatum, but shows significant spatial variability (Howes *et al.*, 2009; Kegeles *et al.*, 2010; McCutcheon *et al.*, 2018a, 2019). Additional advances in our understanding regarding the role of dopamine have also come from findings that striatal dopamine synthesis capacity predicts symptomatic change following treatment with a dopamine antagonist (Jauhar *et al.*, 2018c). In addition, recent in vivo studies using resting state functional magnetic resonance imaging (rMRI) have shown that functional corticostriatal connections can be identified based on the patterns of correlated neural activity (Choi *et al.*, 2012; Jaspers *et al.*, 2017). These developments together allow us to map both corticostriatal connectivity, and spatial variability in striatal dopamine, and to test the hypothesis that variation in dopamine function across the striatum accounts for some of the observed variety in symptoms at an individual level.

In the current study, we use rMRI to map functional corticostriatal connections in individuals presenting with a first episode psychosis, and PET to examine striatal dopamine synthesis capacity within the same individuals. By combining rMRI and PET we were able to evaluate dopamine function within subregions of the striatum that had been defined on the basis of their cortical connectivity at an individual level. We used this data-driven parcellation to examine whether dopamine synthesis capacity within individualised connectivity-defined regions correlated with specific symptoms at baseline assessment, and to improvement in symptoms following treatment with a D_2 receptor antagonist. We hypothesised that distribution of symptom-dopamine relationships across the striatum would reflect the individual topography of cortical projections to the striatum. We focused on auditory hallucinations and motor symptoms as the secondary auditory cortex and motor cortical areas show well circumscribed projections to the striatum, and there are clear apriori potential relationships between symptoms and neurobiological substrate. Specifically, we predicted that both baseline severity and change in severity of hallucinations and of motor symptoms would correlate with dopamine synthesis capacity in striatal regions preferentially connected to auditory and motor cortex, respectively. We also undertook an exploratory analysis to determine whether there were relationships between dopamine function in other striatal regions and other symptom clusters. Finally, we compared our connectivity defined striatal parcellation with published atlas-defined subdivisions (Mawlawi *et al.*, 2001; Martinez *et al.*, 2003), in order to examine whether our individualised connectivity-based method is able to provide additional information over an atlas-based approach.

Materials and methods

Overview

29 first episode psychosis patients and 21 healthy controls received a 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (^{18}F-DOPA) PET scan and an MRI scan at baseline. Clinical ratings were performed at baseline, and for 19 patients ratings were performed for a second time after a minimum of four weeks treatment with a D_2 receptor antagonist (given that this is the period during which most symptomatic response occurs) (Agid *et al.*, 2003; Kahn *et al.*, 2008; Kinon *et al.*, 2010).

For each participant, we used functional connectivity between cortical resting state networks and the striatum to generate individualised connectivity-defined striatal maps. We use these maps to calculate dopamine synthesis capacity for striatal subregions preferentially connected to each cortical network, and investigated whether dopamine function within these different striatal regions showed a relationship with both baseline symptomatology and change in symptoms. The statistical significance of these symptom-dopamine correlations was then tested with a permutation testing approach to investigate the specificity of the observed relationship. The analysis approach is summarised in Figure 1 and described in detail in the Supplement.

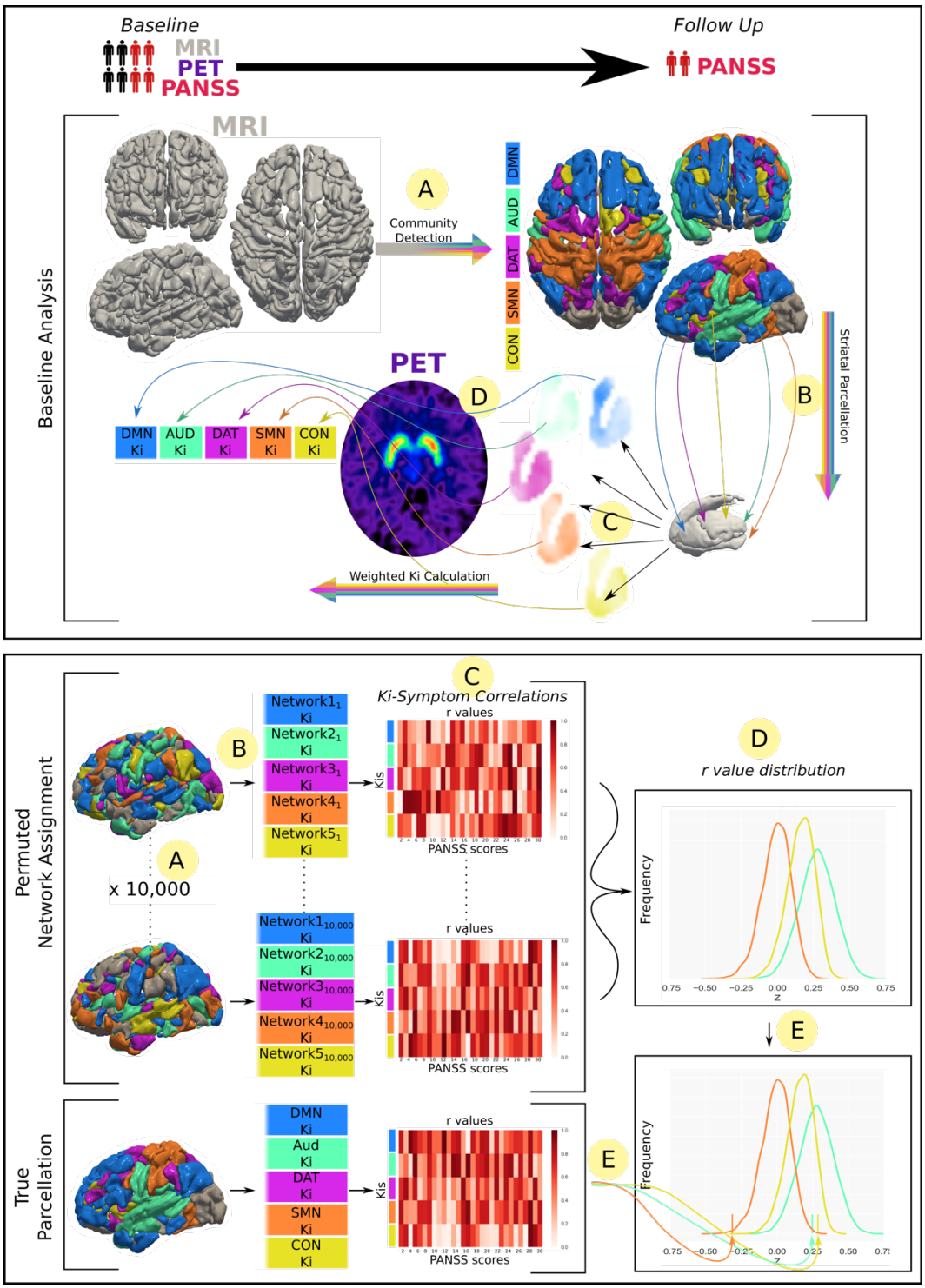

Figure 1. Overview of methods.

(A) Participants receive clinical assessment, MRI and PET at baseline, a subset of patients then received clinical followup. Analysis of baseline data consisted of: (A) Cortical nodes are assigned to networks based on corticocortical resting state functional connectivity (B) Connectivity of each striatal voxel to these cortical networks is calculated (C) Weighted striatal connectivity maps produced for each network (see eFigure 1 and figure 2b) (D) Voxelwise Ki^{cer} maps weighted by these striatal connectivity maps to give a Ki^{cer} value for each network

(B) Significance testing of Ki^{cer}-symptom relationships using a permutation testing approach. In addition to permuting participants (not pictured) cortical ROIs were permuted to generate null distributions: (A)Cortical ROIs shuffled into random networks 10,000 times (B) These shuffled network sets used to calculate Ki^{cer} with the same method described above in 1A (C)Symptom-Ki^{cer} correlations calculated for each null set of Ki^{cer} (D)Null distribution created from repeating step C for each of the null network sets (E) True correlation values compared to null distribution to test statistical significance.

Participants

Participants were experiencing their first episode of a psychotic illness, meeting ICD-10 criteria (World Health Organization, 1992), with no previous illness or treatment episodes, and were either antipsychotic naïve (n=11), antipsychotic free for at least 6 weeks (n=16), or minimally treated for less than 2 weeks (n=2). Age-matched (within 5 years) healthy controls were recruited from the same geographical area through local media advertisements. Controls had no previous or current history of psychiatric illness (assessed by the Structured Clinical Interview for DSM-IV Axis I disorders), no concurrent psychotropic medication use, and no family history of psychosis. See Supplementary Methods and previously published reports for further details regarding recruitment and assessment (Jauhar *et al.*, 2018c, b). A subset of participants subsequently received treatment with a D_2 receptor antagonist or partial agonist and clinical follow up. Change in individual symptoms was quantified as the difference between baseline and follow up scores. Some of the PET data for these participants has been previously reported (Jauhar *et al.*, 2017, 2018b).

Image Acquisition

Participants received a ^{18}F-DOPA pet scan, providing a measure of striatal dopamine synthesis capacity (Kumakura and Cumming, 2009). The cerebellum was used as a reference region, and voxelwise parametric images of dopamine synthesis capacity (K_i^{cer}) were constructed from movement-corrected images using a wavelet-based Patlak approach (Turkheimer *et al.*, 2006). We also determined Ki^{cer} for limbic, associative (the pre- and postcommissural caudate, and precommissural putamen) and sensorimotor (post-commisural putamen) striatal subdivisions, using the atlas-based parcellation approach outlined by Martinez et al. (Martinez *et al.*, 2003). Participants also received a 8.5 minute rfMRI scan on a 3T GE Signa MR scanner. See Supplemental Methods for further details.

Image Analysis – Cortical Network Definition

fMRI signal time series were extracted from the 333 cortical regions (nodes) of the Gordon cortical atlas. Functional connectivity between every pair of nodes was defined as the pairwise z-transformed Pearson correlation coefficients between the fMRI timeseries of each region (Gordon *et al.*, 2016), and was used to define a 333*333 functional connectivity matrix for each participant. The Louvain community detection algorithm was then employed on this whole-cortex connectivity matrix, to group each cortical node into non-overlapping communities in a manner that maximises the modularity of the final network (Blondel *et al.*, 2008). The detected communities corresponded to well recognised resting state networks: the default mode, sensorimotor, cinguloopercular, dorsal attention, auditory and visual networks. The visual network, however, was excluded from subsequent analyses given its relative lack of direct connections with the striatum (Parent and Hazrati, 1995). Analysis was performed with in-house python code freely available at https://github.com/robmcc10/DOPA_symptom.

Image Analysis - Striatal Parcellation and PET Integration

An individual level probabilistic approach was employed. For each participant, for each cortical network identified above, each striatal voxel was assigned a connectivity score between 0 and 1 based on its mean connectivity to all nodes within that network (see eFigure 1). A weighted striatal map was thereby constructed for each of the networks identified. These striatal maps were then overlaid on the PET voxelwise Ki^{cer} maps to enable the calculation of network-specific Ki^{cer} values.

These network-specific Ki^{cer} values were compared with the Ki^{cer} values calculated using the traditional (atlas-based) approach of Martinez et al (Mawlawi *et al.*, 2001; Martinez *et al.*, 2003). Specifically, the correlation coefficients between subdivisions across all participants defined with one method were compared to the correlation coefficients between subdivisions defined using the other method, using a procedure implemented in the R package *cocor* (version 1.1-3) (Silver *et al.*, 2004). This allowed

us to determine whether the connectivity based parcellation led to greater orthogonality between subdivision Ki^{cer}s compared to the Martinez approach.

Dopamine-Symptom Relationships

For both baseline symptoms and change in symptoms, Pearson correlation coefficients were calculated between each item of the Positive and Negative Symptoms Scale (PANSS) and each network-specific Ki^{cer}. We hypothesised that auditory hallucinations (captured in item P3 of the PANSS) would be related to Ki^{cer} within the auditory striatum, and that motor retardation (item G7) would relate to Ki^{cer} within the sensorimotor striatum. Based on previous findings (Abi-Dargham *et al.*, 1998; Demjaha *et al.*, 2012; Jauhar *et al.*, 2018c), we tested the hypothesis that Ki^{cer} would be linearly related to severity of symptoms at baseline, and improvement following treatment with a dopamine antagonist. For hallucinatory behaviour we anticipated a positive correlation, given that previous PET studies have found a positive association between Ki and positive psychotic symptoms (Jauhar *et al.*, 2017), while for motor retardation we anticipated a negative correlation, given that previous PET studies have found markedly reduced Ki^{cer} in individuals displaying motor retardation (Hietala *et al.*, 1995, 1999; Dao-Castellana *et al.*, 1997).

Statistical significance was assessed using two separate permutation testing approaches. First, we permuted participants (10,000 permutations), and compared the correlation coefficients between subregion Ki^{cer} and symptom scores observed in the actual data, with the coefficients observed in the permuted data. This approach, however, does not account for the general relationship between whole striatum Ki^{cer} and total symptoms, in that any significant findings could reflect a general association between whole striatal Ki^{cer} and symptoms in general. Therefore, we also employed a separate approach in which we permuted the cortical nodes assigned to networks (10,000 permutations), thereby creating a null distribution that retained

the relationship with mean striatal dopamine synthesis capacity (Figure 1B). With this approach we were able to test whether our observed subdivision Ki^{cer}-symptom correlation was truly specific to that identified subdivision over and above the general striatal Ki^{cer}-symptom relationships present in the data.

Using these two permutation testing approaches we performed a further analysis in order to account for the collinearity of symptom severity, and further probe the specificity of observed relationships. We employed the same permutation testing approaches, but tested the significance of the correlation coefficient for the symptom in question, *minus* the mean correlation coefficient for all other PANSS items, giving a measure of how strong the observed association was after accounting for the overall relationship with symptoms.

Symptoms were clustered on the basis of between-individual Pearson correlations using the same community detection algorithm used for the clustering of brain regions. This approach clusters subsets of symptoms together so that symptoms in a subset will have a strong association with other symptoms in this subset and a weaker associations with symptoms in other subsets (see supplementary for details)(Blondel *et al.*, 2008). This resulted in symptom clusters that could be described as positive, negative/cognitive, affective, and insight based. In a post-hoc analysis we then examined the relationship between the affective symptoms cluster and 'cingulopercular' striatum Ki^{cer}, and between the negative symptoms cluster and the 'default mode' striatum Ki^{cer}. The same permutation testing approach was taken as when examining single symptoms, and when looking at the difference between the cluster and other symptoms the mean of the coefficients for that cluster was calculated, and the difference score was taken to be this mean cluster score minus the mean of the remaining coefficients. We also examined patient vs. control differences in Ki^{cer} for each striatal subregion using an independent samples t-test.

Data availability

All code used for analysis is freely available at https://github.com/robmcc10/DOPA_symptom. Data is available from the authors upon request.

Results

Participants

50 participants took part in the study (21 controls and 29 patients). Demographic details are given in Table 1. 15 patients had a diagnosis of schizophrenia, 12 of bipolar disorder, one of psychotic depression, and one of delusional disorder. At baseline, mean total PANSS score was 66.7 (SD 20.7). Median time between PET and MRI scan was 15.5 days. 19 of the patients received antipsychotic treatment and clinical follow-up.

Variable	*Controls (N=21)*	*Baseline (N=29)*	*Followup (N=19)*
Male	13(62%)	22(76%)	15(78%)
Age, years	23.3(3.4)	25.5(4.2)	25.1(4.0)
Ethnicity			
% white	14	10	6
% black	2	10	6
% other	5	9	7
Medication status			
Antipsychotic naïve	NA	11	11
Minimally treated[1]	NA	2	2
Antipsychotic free	NA	16	6
PANSS Total	NA	66.7(20.7)	53.5(20.0)
PANSS Positive	NA	17.6(6.9)	12.5(4.7)
PANSS Negative	NA	15.1(6.3)	12.7(5.7)
PANSS General	NA	34.0(10.1)	28.4(11.3)
Injected Activity (MBq)	152.9(12.6)	143.5(7.4)	142.9(9.1)

Table 1 Demographic details of study participants

Data are expressed as n (%) or mean (SD). PANSS=Positive and Negative Syndrome Scale.

[1]Receiving antipsychotic medication for 2 weeks or less

The community detection algorithm assigned nodes to 5 separate networks, these corresponded to well recognised resting state networks; specifically, the default mode (DMN), auditory (AUD), dorsal attention (DAT), sensorimotor (SMN), and cinguloopercular (CON) networks (Figure 1A). The connectivity between these networks and the striatum was calculated at the individual participant level, although for display purposes group averaged maps are shown in Figure 2B.

Baseline Symptom-Dopamine Relationships

Correlation coefficients between baseline symptoms and subdivision Ki^{cer} are displayed in Figure 3A. The relationship between SMN Ki^{cer} and motor retardation (PANSS item G7) was not significant when permuting participants in terms of the G7-SMN Ki^{cer} coefficient itself ($p=0.06$), but was significant when the difference with other symptom-SMN Ki^{cer} correlations was tested ($p=0.009$) (Figure 3). When cortical nodes were permuted this was significant for both the coefficient itself ($p=0.012$), and the difference measure ($p=0.0098$) (Figure 3).

Of those patient reporting hallucinations, all reported that these were solely auditory and not occurring in any other modality. There was no significant relationship between baseline hallucination severity (PANSS item P3) and AUD Ki^{cer} in terms of participant permutations (coefficient only $p=0.09$, difference $p=0.33$), or cortical node permutations (coefficient-only $p=0.43$, difference $p=0.61$).

Symptom Change-Dopamine Relationships

The relationship between SMN Ki^{cer} and change in motor retardation following dopamine antagonist treatment was significant when examined either by permuting participants (coefficient only $p=0.0001$, difference $p<0.0001$), or cortical nodes (coefficient only $p=0.0008$, difference $p=0.0006$) (Figure 4). AUD Ki^{cer} only showed a significant relationship with change in hallucinatory symptoms when examining

participant permutations in terms of the specific item (single item p=0.038, difference p=0.13) (Figure 4).

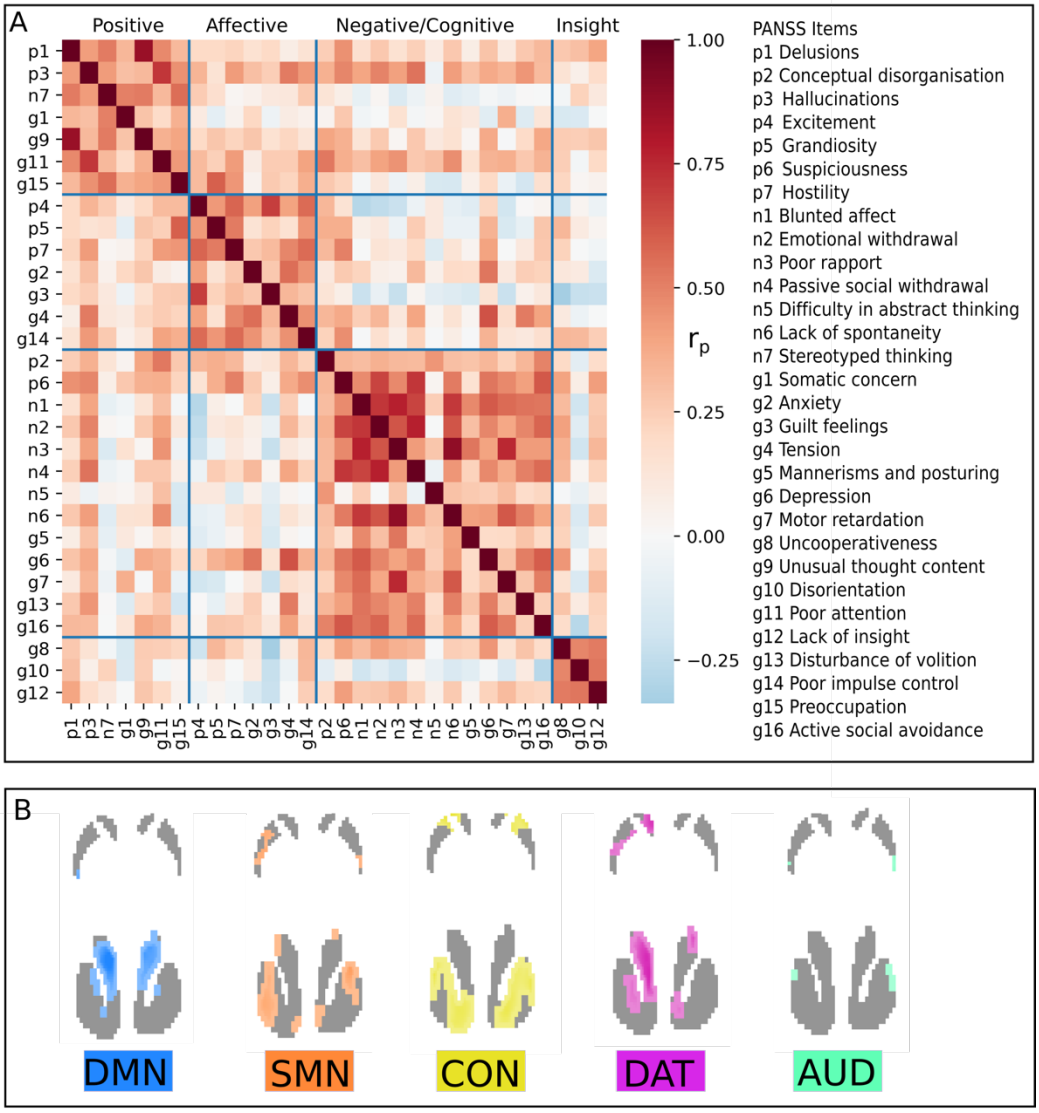

Figure 2. Clustering of psychotic symptoms and connectivity derived striatal maps

A) Heatmap showing the correlations between individual PANSS items, clustered using the Louvain community detection algorithm.

B) Striatal connectivity maps used to weight voxelwise Ki^{cer} maps and generate network specific Ki^{cer}. Greater intensity of colour indicates that a voxel displays greater connectivity to the cortical network in question. The maps are at group level and thresholded for display purposes to show differences between networks more clearly (see efigure for unthresholded maps), while individualised maps were used in practice.

AUD- Auditory , CON – Cingulopercular, DAT – Dorsal attention, DMN – Default mode, SMN – Sensorimotor.

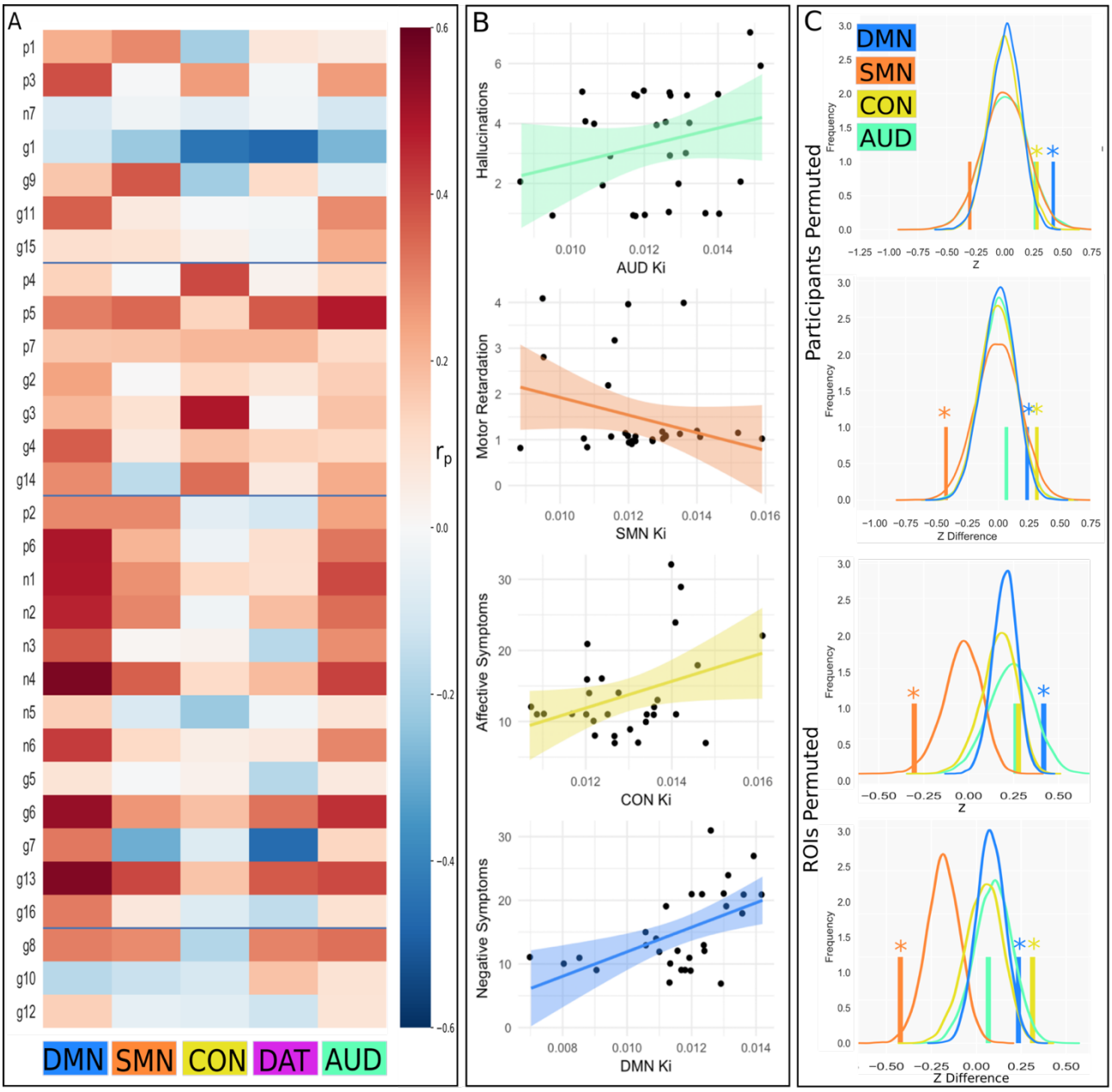

Figure 3. Baseline psychotic symptoms and striatal dopamine

A) Correlations between individual PANSS items and striatal Ki^{cer} across different connectivity defined striatal regions.

B) Specific symptom-Ki^{cer} relationships of interest: AUD and hallucinatory behaviour(p3) where the positive slope indicates greater Ki^{cer} associated with more severe symptoms, SMN and motor retardation (g7), CON and the affective symptom cluster, DMN and the negative symptom cluster.

C) Testing the these symptom-Ki^{cer} relationships: The vertical lines show the position of the observed results. Where the x axis is labelled 'z' this refers to the z-transformed correlation coefficient between the network Ki^{cer} and symptom in question, where it is labelled 'z-difference' this is the z-transformed coefficient in question minus the mean of the other coefficients for that network Ki^{cer}. Curves show a null distribution of z-transformed coefficients created from 10,000 permutations, in (i) this null model is created by permuting individual participants, whereas in (ii) this is achieved by permuting the cortical ROIs from which the striatal maps are derived. * indicates that the difference between the observed result and null distribution is statistically significant at $p<0.05$.

AUD- Auditory , CON – Cingulopercular, DAT – Dorsal attention, DMN – Default mode, SMN – Sensorimotor.

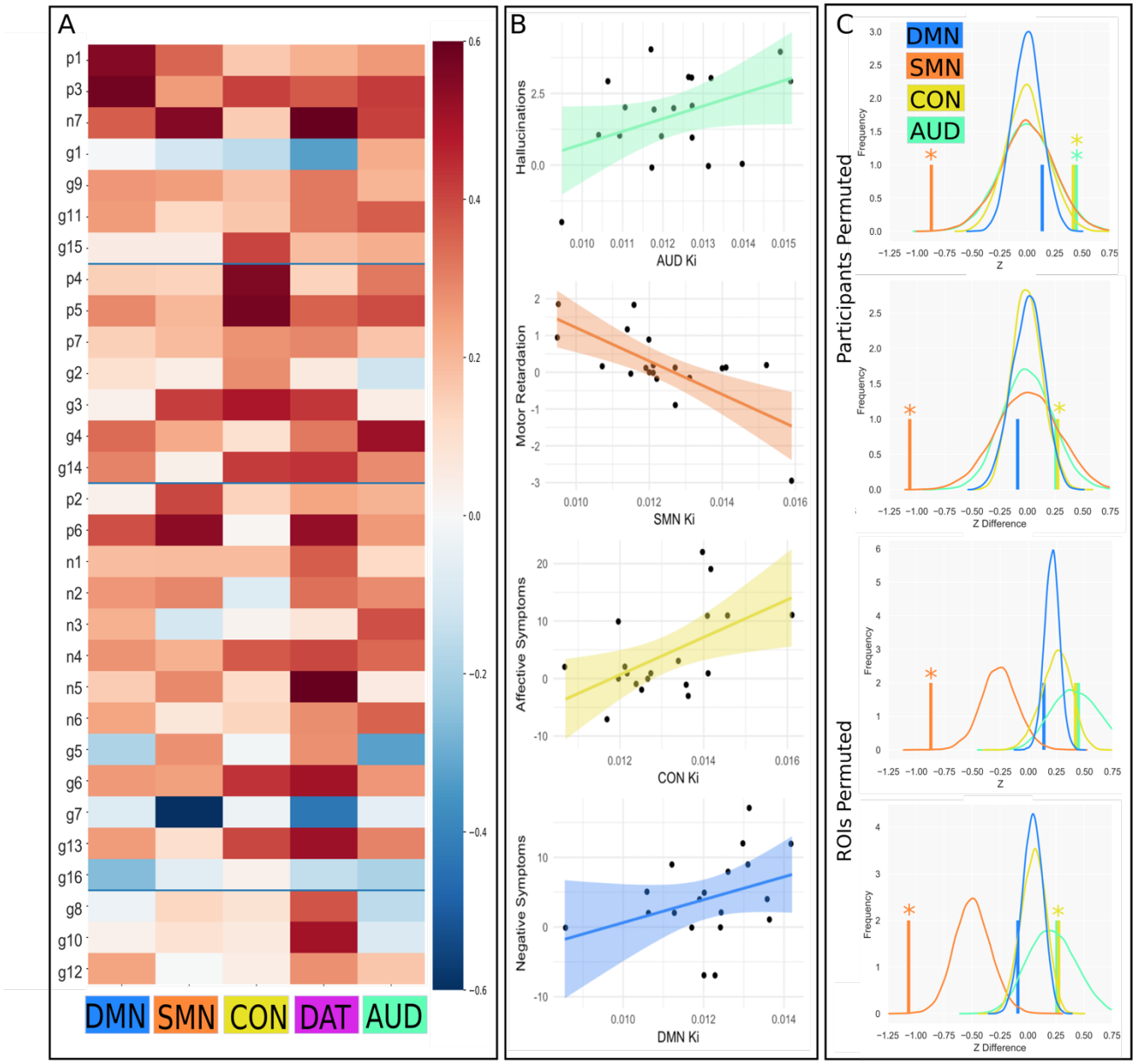

Figure 4. Change in psychotic symptoms and striatal dopamine

A) Correlations between change in individual PANSS items and striatal Ki^{cer} across different connectivity defined striatal regions

B) Specific symptom-Ki^{cer} relationships of interest: Greater baseline AUD Ki^{cer} associated with subsequent improvement in hallucinatory behaviour (p3), SMN and change in motor retardation (g7), CON and change in the affective symptom cluster, DMN and change in the negative symptom cluster.

C) Testing these relationships: The vertical lines show the position of the observed results. Where the x axis is labelled 'z' this refers to the z-transformed correlation coefficient between the network Ki^{cer} and symptom in question, where it is labelled 'z-difference' this is the z-transformed coefficient in question minus the mean of the other coefficients for that network Ki^{cer} (e.g. when examining a single symptom this will be the mean of 29 coefficients). Curves show a null distribution of z-transformed coefficients created from 10,000 permutations, in (i) this null model is created by permuting individual participants, whereas in (ii) this is achieved by permuting the cortical ROIs from which the striatal maps are derived. * indicates that the difference between the observed result and null distribution is statistically significant at $p<0.05$.

AUD- Auditory , CON – Cingulopercular, DMN – Default mode, SMN – Sensorimotor.

Symptom Clusters and Exploratory Analyses

The community detection algorithm assigned PANSS items to four clusters based on the pattern of between-participant symptom correlations (Figure 2A). Clusters corresponded to positive psychotic symptoms (P1, P3, N7, G1, G9, G11, G15), manic/affective symptoms (P4, P5, P7, G2, G3, G4, G14), negative/cognitive symptoms (P2, P6, N1-N6, G5, G6, G7, G13, G16), and insight (G8, G10, G12).

Following visual inspection of the correlation matrices, post hoc exploratory analyses were performed examining for relationships between DMN Ki^{cer} and the negative/cognitive symptom cluster, and CON Ki^{cer} and the affective symptom cluster. The DMN-negative/cognitive relationship was statistically significant when examining both participant permutations (cluster coefficient only $p<0.0001$, difference $p=0.038$) and cortical permutations (cluster coefficient only $p=0.0003$, difference $p=0.026$) (Figure 3). The CON-affective relationship was significant for participant permutations (cluster coefficient only $p=0.029$, difference $p=0.013$), but only for the difference score when cortical permutations were examined (cluster coefficient only $p=0.13$, difference $p=0.0018$) (Figure 3).

Exploratory analyses were again performed examining for symptom change following treatment. The DMN-negative/cognitive relationship was not statistically significant for any tests (participant permutation item only $p=0.14$, difference $p=0.32$, cortical node permutation item only $p=0.86$, difference $p=0.92$) (Figure 4). The CON-affective relationship was significant for participant permutations (item only $p=0.013$, difference $p=0.028$) and for the difference score when cortical nodes were permuted (item only $p=0.091$, difference $p=0.0156$) (Figure 4).

A post-hoc analysis of patient vs. control differences between subdivisions showed the greatest difference between groups was within the DMN subregion, although this did not reach statistical significance ($t=1.72$, $p=0.09$) (eFigure 3).

Comparison with Existing Parcellation Methods

Orthogonality between subdivision Ki^{cer}s is required for the investigation of unique subdivision-symptom relationships, as a high degree of correlation between subdivisions effectively prevents individual subdivision relationships from being studied. We analysed orthogonality in our individualised connectivity-based topographical approach and that in a group based approach that used the atlas-based striatal subdivisions from Martinez et al. (Mawlawi *et al.*, 2001; Martinez *et al.*, 2003). Correlations between subdivisions showed that the Martinez subdivisions showed a highly collinear relationship (r_p=0.76-0.92), while in contrast the individualised connectivity defined subdivisions showed much greater orthogonality (r_p 0.23-0.65) that was statistically significant for 26 of 30 possible comparisons between the methods (eFigure 2).

Discussion

We found that the relationship between dopamine function and symptoms varied according to the striatal region examined. Specifically, dopamine function in striatal regions linked to sensorimotor cortex was associated with both the severity of motor retardation pre-treatment, and the change in motor retardation following treatment with a dopamine antagonist. There was no clear association, however, between hallucination severity and dopamine function within striatal regions linked to the auditory cortex. In the exploratory analysis, dopamine function within the striatal regions linked to the default mode network, and cingulopercular network were associated with negative/cognitive and affective symptoms respectively.

While previous studies have investigated the relationship between striatal dopamine function and symptoms in psychotic disorders, this has predominantly been at the level of the whole striatum, and so has not addressed the question of specific symptom-subregion relationships (Abi-Dargham *et al.*, 1998; Demjaha *et al.*, 2012; Jauhar *et al.*, 2018c). Although more recent studies have examined subdivisions, the typical approach employed precludes investigation of the current hypothesis due to the high degree of collinearity between subdivisions. We demonstrated significantly greater orthogonality in our individualised connectivity-based approach, which allowed, for the first time to our knowledge, specific subregion-symptom relationship to be investigated. Moreover, in contrast to our data-driven parcellation approach, traditional atlas-based subdivisions do not take into account the likely considerable spatial variability in striatal functional specificity that occurs between participants. Finally, our permutation testing approach is robust to outliers and skewed data distributions, and allowed for thorough testing of the specificity of relationships both in terms of symptoms and subregions.

Although motor symptoms are frequently ascribed to antipsychotic drug treatment, recent studies show that around 20% of antipsychotic naïve first episode patients are affected by motor signs and symptoms (Pappa and Dazzan, 2009). Furthermore, historical descriptions of schizophrenia prior to the use of antipsychotic drugs were almost unanimous in their inclusion of motor signs as a core feature of the syndrome (Kendler, 2016). We found that a lower Ki^{cer} within striatal regions linked to sensorimotor cortex was associated with more severe motor retardation. Interestingly, this lower Ki^{cer} also predicted a greater improvement following antipsychotic treatment. One would not necessarily expect a symptom purportedly driven by low presynaptic dopamine to respond to treatment with a dopamine antagonist. It may therefore be the case, that the relationship observed with treatment may represent a regression to the mean, although a placebo arm would be required to definitively test this.

We found no specific relationship between Ki^{cer} in the auditory regions of the striatum and the severity of hallucinatory behaviour. This may be taken to suggest that auditory hallucinations are not directly driven by dopaminergic dysfunction within this specific region, and are associated with either more general dopaminergic dysfunction across the striatum, or potentially a non-dopaminergic mechanism (Howes *et al.*, 2013). Alternatively, the lack of a finding may conceivably be secondary to the noise inherent in both imaging and symptom measures. The fact that a small number of patients were receiving antipsychotic treatment at the time of scan is unlikely to have had a significant impact given that this does not appear to affect presynaptic dopamine function (Jauhar *et al.*, 2019).

The default mode network predominantly mapped onto striatal areas that have been defined as 'associative' based on their connection to cortical regions broadly involved in cognition (Martinez *et al.*, 2003). Dopamine dysfunction within this region showed an association with the severity of negative/cognitive symptoms. Recent work has

suggested mechanisms via which excessive striatal dopamine transmission may underlie these symptoms through a range of mechanisms including impairments in probabilistic learning and disrupting corticostriatal connectivity (Maia and Frank, 2017; McCutcheon *et al.*, 2019). While it can be hard to determine in first episode cohorts whether negative symptoms are secondary to positive symptoms, the symptom clustering approach we adopted mitigates against this by maximising the orthogonality of symptom clusters. A previous PET study found a relationship between greater negative symptom severity and *reduced* synaptic dopamine levels (Kegeles *et al.*, 2010), differences here may result from marked differences in experimental technique, and the fact that the current study included only first episode patients while the other cohort mostly consisted of chronically ill patients. The cinguloopercular network mapped onto regions of the striatum that predominantly receive projections from limbic cortical regions such as the medial prefrontal cortex and anterior cingulate (Martinez *et al.*, 2003). The association observed with affective symptoms therefore, is in keeping with research linking this part of the striatum to affective processing (Price and Drevets, 2012). However, given the exploratory nature of these analyses, we suggest these findings warrant further testing in new cohorts.

As reported previously, we did not observe a significant difference between patients and controls in terms of striatal Ki^{cer} (Jauhar *et al.*, 2018b). This may represent a type II error, possibly exacerbated by the fact that our cohort included a number individuals that were nonresponsive to antipsychotic treatment, a characteristic associated with normal Ki^{cer} (Demjaha *et al.*, 2012; Jauhar *et al.*, 2018c). Nevertheless, this raises a more general question as to how one characterises 'aberrant' dopamine function, given that even in responsive individuals there is substantial overlap between patient and control Ki^{cer} values.

Previous PET studies looking at striatal subdivisions in schizophrenia have used a group template based approach when parcellating the striatum (Howes *et al.*, 2009; Kegeles *et al.*, 2010; Mizrahi *et al.*, 2012). This may be inappropriate given that wide variety that is apparent in terms of striatal volume, shape, and anatomical connectivity (Chakravarty *et al.*, 2015; Levitt *et al.*, 2017). An individualised connectivity based approach may also be preferable given that schizophrenia is associated with altered functional corticostriatal connectivity (McCutcheon *et al.*, 2019), and the fact that both corticostriatal (Kim *et al.*, 2018), and corticocortical connectivity (McCutcheon *et al.*, 2018b), appear to show a relationship with striatal dopamine function. The individualised approach we employed may potentially account for some of these differences. We used a probabilistic (as opposed to a winner-takes-all) approach in our main analyses given the fact that although corticostriatal pathways run in parallel there is a high degree of overlap (Haber, 2016).

Future work would benefit from more detailed behavioural assessment, and larger sample sizes. Obtaining PET and MRI measures simultaneously with combined PET-MR may improve the signal-to-noise ratio. It would also be of interest to explore alternative methods for parcellating the striatum, both using resting state data (Barnes, 2010; Jaspers *et al.*, 2017), but also mapping anatomical connectivity using diffusion tensor imaging (Tziortzi *et al.*, 2011).

In conclusion, we show that motor retardation associated with schizophrenia are specifically linked to dopamine dysfunction within regions of the striatum linked to the sensorimotor cortex. We also find some evidence to suggest that dopamine dysfunction within striatal regions linked to the default mode network, and cingulopercular network may relate to negative and affective symptoms respectively.

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Competing interests

ODH has received investigator-initiated research funding from and/or participated in advisory/ speaker meetings organised by Astra-Zeneca, Autifony, BMS, Eli Lilly, Heptares, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. Neither Dr Howes or his family have been employed by or have holdings/ a financial stake in any biomedical company. M.M. has consulted for Cambridge Cognition, Lundbeck and Forum Pharmaceuticals in the past 3 years. He has also received research funding from Takeda, Eli Lilly and Roche. The other authors declare no conflicts of interest.

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Supplementary Information

Methods

Ethics

This study was approved by the East of England-Cambridge East NHS Research Ethics Committee, and Administration of Radioactive Substances Advisory Committee (ARSAC). All participants provided informed written consent to participate.

Participants – Recruitment

Participants were recruited from clinical services in South and West London. Exclusion criteria for all subjects were: history of significant head trauma, dependence on illicit substances, medical co-morbidity (other than minor illnesses), lifetime use of antipsychotic drugs for longer than two weeks,[1] contra-indications to PET and MRI scanning (such as pregnancy), or prescription of mood stabilizer medication.

Participants – Clinical Assessment

Participants were classified by antipsychotic exposure as antipsychotic naïve, antipsychotic free (prior oral antipsychotic medication but free of treatment for at least 6 weeks (oral) or 6 months (depot, if relevant)) or minimally treated (taking antipsychotic medication for two weeks or less).

Participants were assessed using the Positive and Negative Syndrome Scale (PANSS)[2] at baseline and after taking a dopamine antagonist at a therapeutic dose (as specified by the Maudsley Prescribing Guideline)[3] for a minimum of four weeks. To assess antipsychotic concordance we used required evidence of good adherence from at least two of the following sources: antipsychotic blood plasma levels, pharmacy and electronic medical dispensing records, patient report and independent

source report (e.g. carer). Subjects were required to have taken at least 80% of prescribe doses.[4]

Choice of antipsychotic medication was at the discretion of the treating clinician as per usual clinical care.

18F-DOPA PET Data Acquisition and Analysis

Participants were not permitted to smoke or consume caffeine for four hours preceding the scan. After acquiring a CT scan for attenuation correction, PET images were acquired using a Siemens Biograph HiRez XVI PET scanner (Siemens Healthcare, Erlangen, Germany) at Imanova Centre for Imaging Sciences.

One hour prior to scanning, participants received 400mg entacapone and 150mg carbidopa, to prevent formation of radiolabelled metabolites and reduce peripheral metabolism. Approximately 160 MBq of ^{18}F-DOPA was administered by bolus intravenous injection. The quantification pipeline was consistent with previous works.[5] Correction for head movement during the scan was performed by denoising the non- attenuation-corrected dynamic images using a level 2, order 64 Battle-Lemarie wavelet filter. Frames were realigned to a single reference frame, acquired 20 minutes post-injection, employing a mutual information algorithm.[6,7] The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images, creating a movement-corrected dynamic image, which was used in the analysis. Realigned frames were then summated to create an individual motion-corrected reference map for the brain tissue segmentation. The cerebellum was used as a reference region, and K_i^{cer} was calculated with the Patlak-Gjedde graphical approach adapted for reference tissue input function.[8] Image processing and quantification was done using in-house code with MATLAB 2012b.

In order to generate the voxelwise Ki maps we implemented a previously established method[9] in which Ki^{cer} parametric images of the brain were constructed from motion-corrected images using a wavelet-based approach.[10] The parametric image for each participant was then normalized into Montreal Neurological Institute standard space (matrix dimension: 91x109x91; voxel size: 2mm isotropic) using the participant's PET summation image and the ^{18}F-DOPA template.

MRI Acquisition and Preprocessing

Participants also received a rfMRI scan on a 3T GE Signa MR scanner. Functional imaging consisted of T2* weighted echo planar image slices. 256 volumes were acquired, consisting of 39 interleaved slices (3.5 mm slice thickness, 3.75 mm x 3.75 mm voxel dimensions in plane) with a repetition time (TR) of 2000 ms, and echo time (TE) 30 ms. A structural image was obtained using a gradient-echo scan (TR=7.0s, TE=2.8s, flip angle=11°, in plane resolution=1mm x 1mm, slice thickness=1.2mm, 196 slices).

Image pre-processing was performed using a standard pipeline implemented in the CONN toolbox (version 17.b)[11] for Statistical Parametric Mapping software (SPM 12 (6906)). A standard preprocessing pipeline was used consisting of slice timing correction, realignment, and normalisation to MNI space. Images were smoothed with a Gaussian kernel of 8mm full-width-half-maximum. The ART toolbox was used to account for motion and artefact detection using anatomical component based correction (aCompCor) of temporal confounds relating to head movement and physiological noise. This method models noise effects at a voxel level based on estimates derived from principal components of noise regions of interest (white matter and CSF, eroded by one voxel to minimise partial volume effects), and then removes these from the BOLD timeseries using linear regression. Six residual head motion parameters and their first order temporal derivatives were also entered as regressors into the first level model. A confounding effect accounting for

magnetisation stabilisation, and its first order derivative was entered. Artifact/outlier scans (average intensity deviated more than 5 standard deviations from the mean intensity in the session, or composite head movement exceeded 0.9 mm from the previous image) were also regressed out. Preprocessed data were temporally bandpass filtered (0.008-0.09 Hz)

Cortical Network Assignment

Time series were extracted from the 333 nodes of the Gordon atlas.[12] The Gordon parcellation was developed using resting state boundary maps observed in a sample of 120 healthy young adults, it shows superior within parcel homogeneity in comparison to other parcellations, making it an appropriate choice for the analysis of resting state data. For each participant, a 333x333 connectivity matrix (also termed a *graph*) was constructed where each edge between two nodes represents the z-transformed Pearson correlation coefficient of the time series of these nodes.

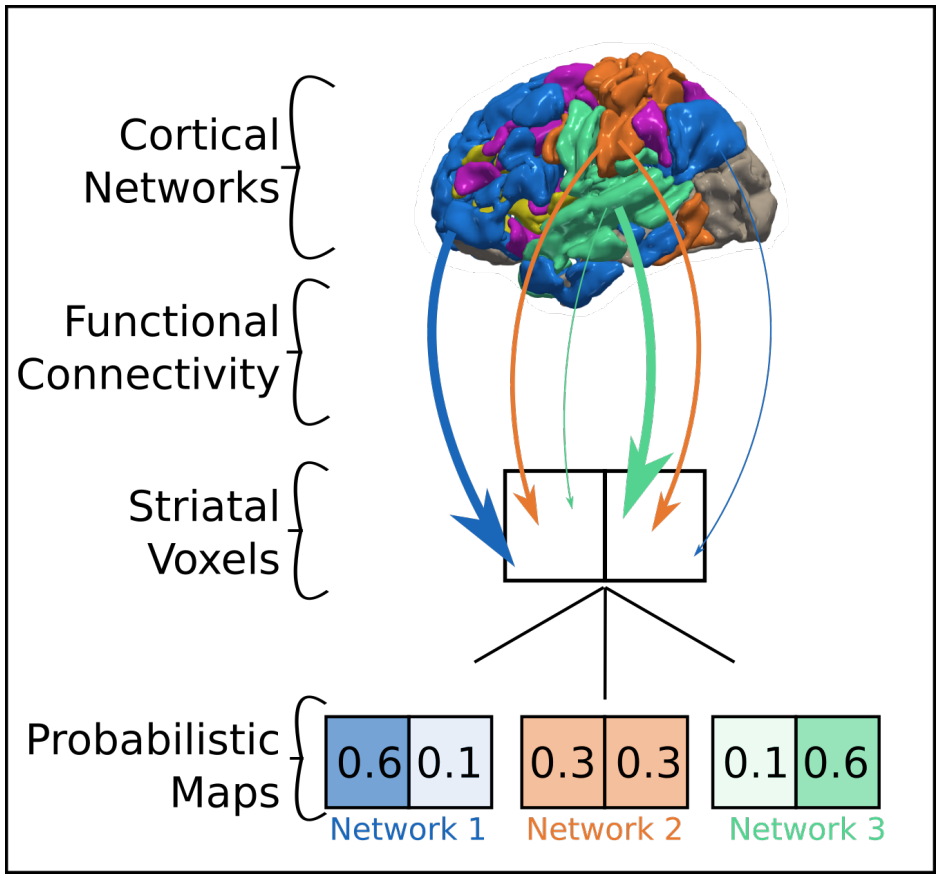
A group averaged connectivity matrix was then constructed. To do this we first rescaled each participant's graph, by subtracting from each edge that participant's average edge strength, and dividing by the standard deviation of the strength of all that graph's edges. As a result, all participants then have the same average graph connectivity strength, and so individuals with greater average connectivity values do not have undue topological influence.

We then ran the Louvain community detection algorithm on the whole brain group level graph,[13] in order to assign individual nodes to networks based on the connectivity patterns present in the current dataset. Due to the non-deterministic nature of the Louvain algorithm, a previously described consensus clustering approach was employed.[14,15] Edges between nodes closer than 10mm were discarded (euclidean minimum distance between two closest points of the two nodes), and the strongest 5% of edges were retained and binarised. The gamma parameter was set

to 1.4. Networks defined by this approach were then labelled according to whichever of the apriori defined networks they showed the greatest overlap with, as quantified with the Sorensen-dice coefficient.[12,16] This identified the default mode, sensorimotor, cinguloopercular, dorsal attention, auditory and visual networks. The visual network was excluded in subsequent analysis given its relative lack of direct connections with the striatum.[28]

Striatal Probabilistic Parcellation

For each striatal voxel the z-transformed correlation coefficient between the voxel and the 333 Gordon nodes was calculated. Then for each of the networks defined in the *Network Assignment* step above the mean connectivity of that network's nodes to the voxel was calculated, with negative values being set at 0. When this had been performed for each network these values were then scaled so that at each voxel the sum of the five connectivity values (one for each network) equalled 1 (see eFigure 1).



eFigure 1. Striatal probabilistic connectivity maps

Each striatal voxel is assigned a value for each of the cortical networks, based on the mean connectivity of the voxel to each node in the network. The total connectivity score for each voxel sums to 1. In the example above the left hand voxel shows is weighted most strongly for network 1, and least for network 3.

PET-MRI Integration

At the individual participant level for each network, for each striatal voxel we multiplied the Ki^{cer} value at that voxel by the weight assigned to that network at that voxel. We then averaged across voxels to generate for each network a network specific Ki^{cer}.

Results

Cortical network assignment

Numbers refer to the node labels provided by Gordon et al.[12]

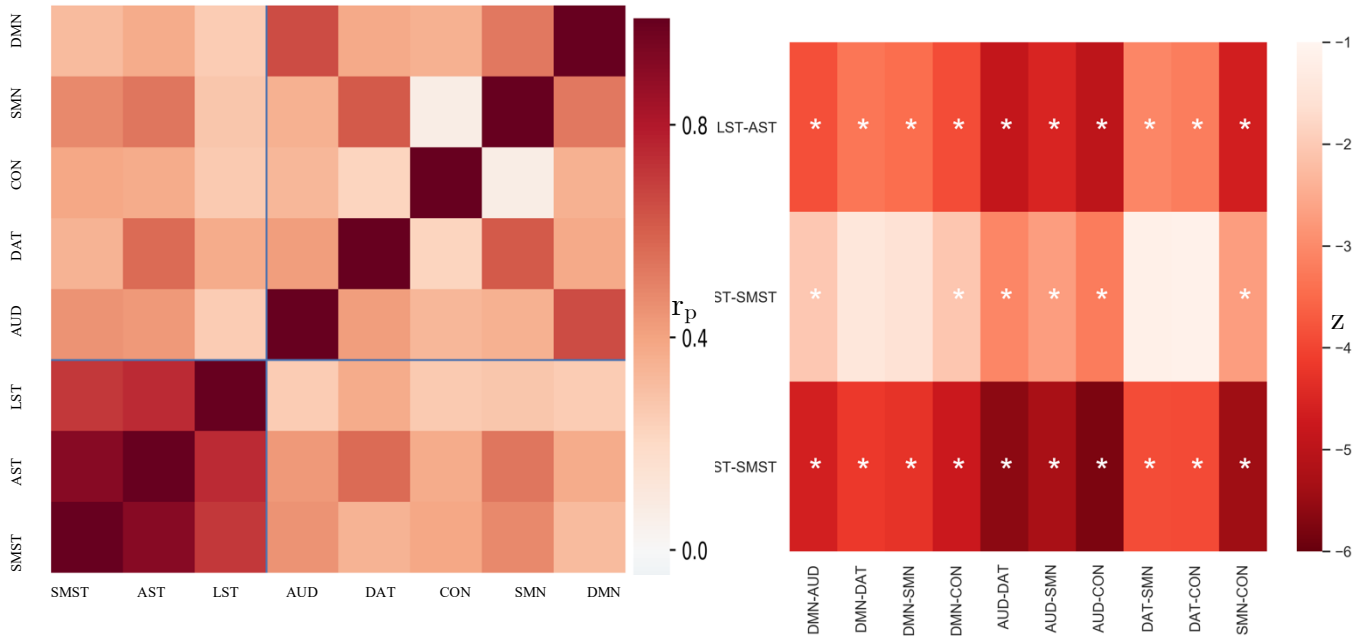
Default Mode = [7, 124, 162, 165, 167, 168, 170, 182, 186, 200, 219, 220, 225, 236, 237, 240, 241, 242, 247, 250, 253, 259, 260, 261, 267, 272, 273, 275, 276, 277, 278, 279, 283, 289, 290, 291, 301, 302, 315, 316, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 1, 4, 6, 9, 23, 24, 25, 44, 60, 61, 75, 78, 79, 85, 86, 94, 96, 107, 108, 109, 113, 114, 115, 116, 126, 127, 128, 129, 133, 145, 146, 148, 150, 151, 156, 157, 158, 159, 221, 292, 300, 333]

Sensorimotor = [2, 30, 31, 32, 33, 35, 36, 37, 38, 45, 46, 47, 50, 51, 54, 55, 56, 57, 58, 104, 163, 180, 190, 191, 193, 194, 195, 201, 202, 203, 204, 205, 206, 209, 210, 211, 213, 214, 215, 216, 217, 251, 252, 262, 265, 270]

Auditory = [3, 10, 22, 39, 53, 59, 62, 65, 66, 67, 68, 69, 70, 71, 72, 76, 77, 81, 101, 102, 103, 105, 111, 160, 161, 164, 171, 196, 197, 212, 218, 222, 223, 224, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 238, 239, 244, 246, 268, 269, 271, 274, 329, 330, 331, 332, 64]

Cingulopercular = [12, 21, 26, 27, 28, 29, 80, 82, 83, 84, 117, 147, 149, 152, 153, 181, 183, 184, 185, 187, 188, 192, 243, 245, 248, 249, 317, 318]

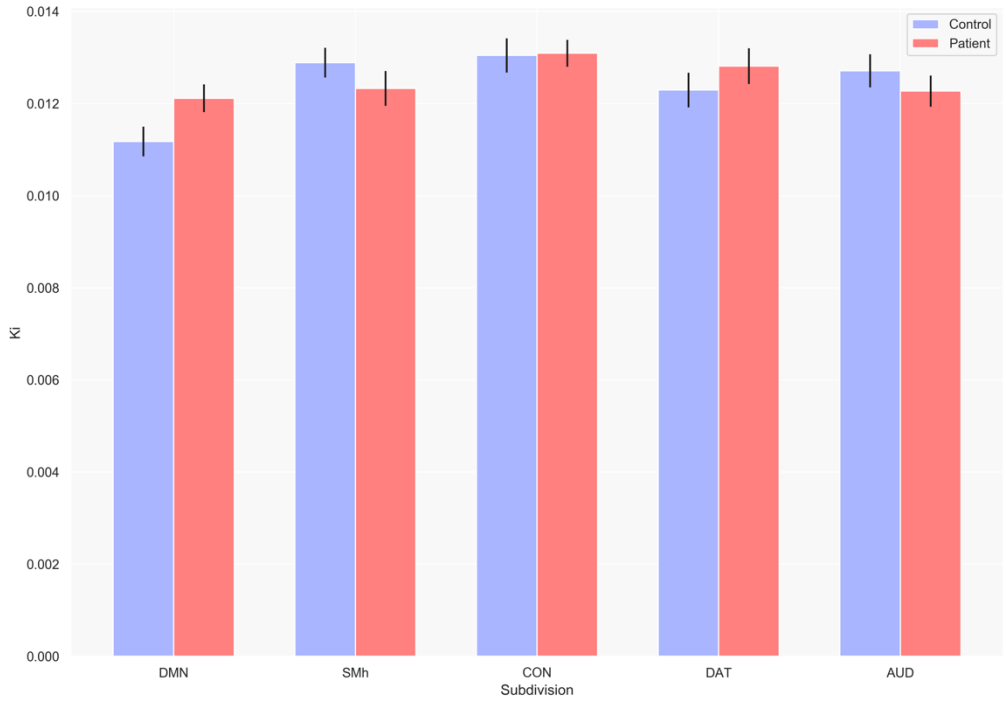
Dorsal Attention = [34, 40, 41, 42, 43, 48, 49, 87, 91, 92, 93, 95, 154, 155, 189, 198, 199, 207, 208, 257]



eFigure 2. Comparison of connectivity derived and Martinez subdivisions

A) Heatmap displaying correlation coefficients between Ki^{cer} values for different subdivisions. There is greater orthogonality between resting state defined subdivisions (r_p = 0.23-0.65) compared to subdivisions defined using the method of Martinez et al divisions(r_p0.71-0.92).

B) Comparing the magnitude of these intra-method correlation coefficients, these are significantly lower (*= $p<0.05$) for the resting state method (i.e indicating greater orthogonality) for all but 4 of the 30 comparisons.



eFigure 3. Comparison of patients and controls resting state defined Ki^{cer}

Average dopamine synthesis capacity within resting state defined striatal subdivisions for patients and controls. There were no significant differences between the groups for any of the subdivisions. Error bars = +/- 1SE.

AUD- Auditory , CON – Cingulopercular, DAT – Dorsal attention, DMN – Default mode, SMN – Sensorimotor.

eFigure 4. Striatal connectivity maps used to weight voxelwise Kicer maps and generate network specific Ki^{cer}.

AUD- Auditory , CON – Cingulopercular, DAT – Dorsal attention, DMN – Default mode, SMN – Sensorimotor.

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5. Chronic psychosocial stressors are associated with alterations in salience processing and corticostriatal connectivity

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Abstract

Psychosocial stressors including childhood adversity, migration, and living in an urban environment, have been associated with several psychiatric disorders, including psychotic disorders. The neural and psychological mechanisms mediating this relationship remain unclear. In parallel, alterations in corticostriatal connectivity and abnormalities in the processing of salience, are seen in psychotic disorders. Aberrant functioning of these mechanisms secondary to chronic stress exposure, could help explain how common environmental exposures are associated with a diverse range of symptoms. In the current study, we recruited two groups of adults, one with a high degree of exposure to chronic psychosocial stressors (the exposed group, n=20), and one with minimal exposure (the unexposed group, n=22). All participants underwent a resting state MRI scan, completed the Aberrant Salience Inventory, and performed a behavioural task – the Salience Attribution Test (SAT). The exposed group showed reduced explicit adaptive salience scores (cohen's d= 0.69, p=0.03) and increased aberrant salience inventory scores (d=0.65, p=0.04). The exposed group also showed increased corticostriatal connectivity between the ventral striatum and brain regions previously implicated in salience processing. Corticostriatal connectivity in these regions negatively correlated with SAT explicit adaptive salience (r=-0.48, p=0.001), and positively correlated with aberrant salience inventory scores (r=0.42, p=0.006). Furthermore, in a mediation analysis there was tentative evidence that differences in striato-cortical connectivity mediated the group differences in salience scores.

Key Words: stress, schizophrenia, psychosis, striatum, corticostriatal, functional connectivity

Introduction

Several environmental factors that can be considered chronic psychosocial stressors are associated with an increased risk of developing schizophrenia, but the psychological and neurobiological mechanisms mediating this increased risk remain incompletely understood (Tost et al., 2015). Disruption in salience processing has been proposed as a central deficit in schizophrenia, whereby the 'salience' of a stimulus refers to the significance that stimulus holds for an organism (Winton-Brown et al., 2014). Corticostriatal circuits play an important role in salience processing, and disruption of these circuits is seen in schizophrenia (Dandash et al., 2014; Fornito et al., 2013; Levitt et al., 2017). In the current study, we investigated whether exposure to chronic psychosocial stressors was associated with alterations in salience processing, and whether this was linked to changes in corticostriatal connectivity.

1.1 Chronic Psychosocial Stressors and Schizophrenia

Many of the environmental risk factors associated with schizophrenia can be broadly conceptualised as chronic psychosocial stressors (Selten and Cantor-Graae, 2005; Walker and Diforio, 1997). These include childhood adversity, migration, and urbanicity (De Loore et al., 2007; Howes et al., 2017; Linscott and van Os, 2013; Morgan et al., 2009; Van Nierop et al., 2014).

Studies investigating the influence of these psychosocial stressors upon brain function have typically investigated one factor at a time, despite the fact that the risk factors cluster together and potentially share common underlying mechanisms (Hjern et al., 2004; Morgan et al., 2007; Wicks et al., 2005). Moreover, epidemiological evidence suggests that there are at least additive, and potentially synergistic effects between risk factors (Guloksuz et al., 2015; Lataster et al., 2012; Morgan et al., 2014; Schafer and Fisher, 2011).

Several lines of evidence have suggested links between psychosocial stress exposure and alterations in salience processing. The finding that individuals exposed to early life trauma show evidence of both blunted responses to reward, and increased rates of psychotic experiences, suggests that alterations to both adaptive and aberrant salience processing mechanisms may be present (Croft et al., 2018; Hanson et al., 2016). Given the central role of dopamine in salience processing, the finding that presynaptic dopamine function appears altered in immigrants and individuals that have suffered childhood adversity, suggests that this may be a mechanism via which these exposures lead to an increased risk of mental illness (Egerton et al., 2017, 2016; Howes et al., 2017; Pruessner et al., 2004). There is also evidence that exposure to chronic psychosocial stressors is associated with functional alterations in brain regions involved in salience processing (Akdeniz et al., 2014; Lederbogen et al., 2011; McCutcheon et al., 2018; Teicher et al., 2016), and to corticostriatal functional connectivity (Hanson et al., 2018; Hart et al., 2018). (Hanson et al., 2018; Hart et al., 2018). Taken together this suggests that psychosocial stress exposure could lead to cortico-striatal dysfunction and aberrant salience processing (figure 1). To our knowledge, however, none have directly investigated how these functional alterations relate to salience processing.

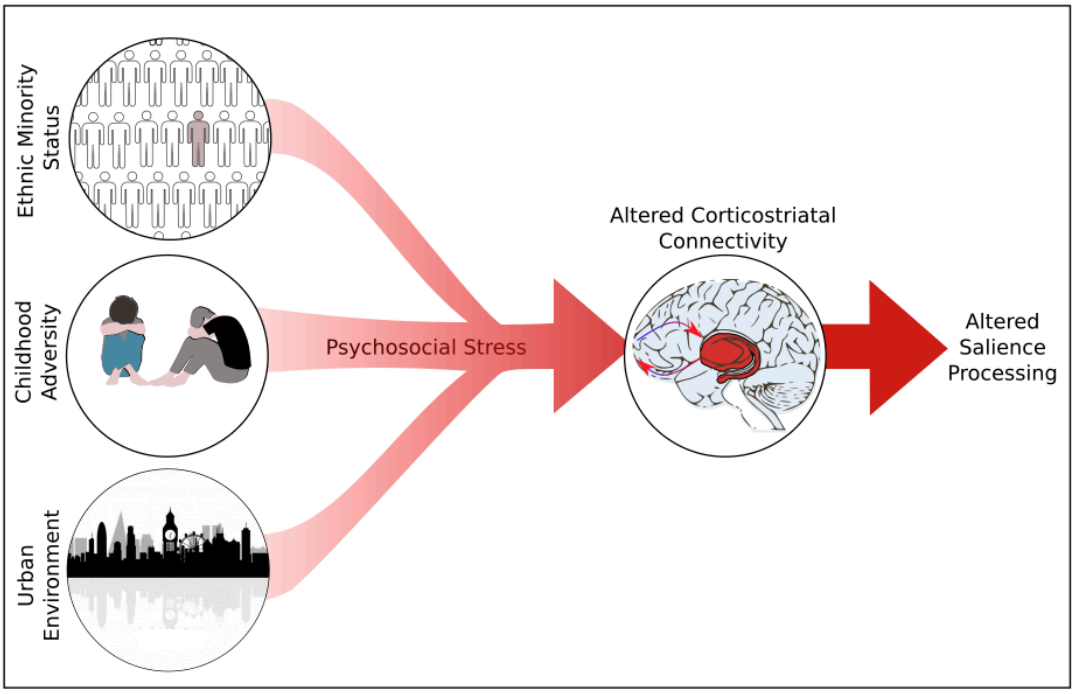


Figure 1. Proposed pathway in which exposure to chronic psychosocial stressors leads to alterations in corticostriatal connectivity and subsequent alterations in salience processing.

1.2 Salience Processing

The Salience Attribution Test (SAT) was developed to quantify four related aspects of salience processing. The test involves participants responding to a probe that is paired with stimuli that vary across two dimensions (e.g. colour and shape). One of these dimensions is associated with a greater probability of reward (the outcome relevant dimension), whereas the other bears no relationship to the likelihood of reward (the outcome irrelevant dimension). The test has both implicit (based on reaction times) and explicit (based on participant ratings) components, and measures both aberrant (the extent to which the irrelevant dimension is thought to signal outcome) and adaptive salience (the extent to which relevant dimension is thought to signal to outcome).

Studies using the SAT in individuals with schizophrenia, have consistently demonstrated reduced adaptive salience compared to healthy controls (Abboud et al., 2016; Katthagen et al., 2016; Pankow et al., 2016; Roiser et al., 2009). Differences in aberrant salience have also been observed (Katthagen et al., 2016; Pankow et al., 2016), but this is not a consistent finding (Abboud et al., 2016; Roiser et al., 2009). Abnormal salience processing has also been demonstrated in those at high clinical risk of psychosis, and in dependent cannabis users (Bloomfield et al., 2016; Roiser et al., 2013); while in healthy controls greater dopamine synthesis capacity within the ventral striatum has been linked to higher aberrant salience scores (Boehme et al., 2015). The aberrant salience inventory (ASI) is a questionnaire that was developed in order to quantify the subclinical phenomenology of disrupted salience processing (Cicero et al., 2010). Inventory scores are related to measures of psychosis proneness (Cicero et al., 2010), and have been found to be increased in individuals treated with dopamine agonists (Poletti et al., 2014).

Several neural systems are involved in salience processing. Schultz et al. demonstrated that mesostriatal dopamine neurons play a role in assigning value to stimuli, based on accompanying rewards (Schultz et al., 1997), while more recent research has shown that these neurons respond to surprising stimuli even in the absence of any change in value, suggesting that their role extends beyond value encoding to include signalling the salience of relevant stimuli in general (Takahashi et al., 2017; Winton-Brown et al., 2014). In concert with the role that striatal dopamine plays, cortical regions are also involved in the propagation of salience signals. Appropriate functioning of corticostriatal connections is necessary for the successful integration of these cortical networks and striatal dopamine signalling (Menon and Uddin, 2010; Peters et al., 2016; Roiser et al., 2010), and disruption of corticostriatal connectivity has been observed in disorders of salience processing

such as schizophrenia (Dandash et al., 2014; Fornito et al., 2013; Levitt et al., 2017).

In the current study, we recruited individuals with a history of either high or low exposure to chronic psychosocial stressors. Corticostriatal connectivity was measured using resting state functional magnetic resonance imaging (rfMRI), and participants also completed the Aberrant Salience Inventory and undertook the SAT. We hypothesised that exposed individuals would display increased aberrant, and reduced adaptive salience scores. We also hypothesised that exposed individuals would display alterations in corticostriatal functional connectivity, and that these alterations would be related to alterations in salience processing.

Materials and Methods

2.1 Participants

Two groups of healthy volunteers were recruited, one that had been exposed to chronic psychosocial stressors (the 'exposed' group), and one with minimal exposure (the 'unexposed' group). Two groups of healthy volunteers were recruited, one that had been exposed to chronic psychosocial stressors (the 'exposed' group), and one with minimal exposure (the 'unexposed' group). Participants were recruited via online, leaflet and newspaper advertising. Online and newspaper advertising was performed in both and rural areas, while leaflet advertising was performed only in rural areas. Group assignment was made only following the screening interview. All subjects were aged 18-45, had no personal history of psychiatric illness, and no family history of psychotic illness. All participants provided written informed consent, and the study had research ethics committee permission.

The exposed group had exposure to at least two of the following three risk factors: Childhood and current dwelling in urban environment (defined as local authority population density >50 persons/hectare); a history of 1st or 2nd generation migration; and a history of childhood adversity before age 16 years, which was defined as a Childhood Trauma Questionnaire subscale (physical, emotional, or sexual abuse) classification score of "moderate/severe" or "severe/ extreme" (Bernstein et al., 2003). The unexposed group currently lived in a non-urban area, had no history of urban living for longer than six months, had no history of migration, and no history of childhood adversity.

Population density of current dwelling was obtained from the 2011 census (Office of National Statistics, 2011). Questionnaires conducted included the Aberrant

Salience Inventory (ASI) (Cicero et al., 2010), and the Childhood Trauma Questionnaire (Bernstein et al., 2003).

2.2 Salience Attribution Test

Aberrant and adaptive salience was measured using the SAT (Roiser et al., 2009). This is a task in which participants are presented with an image from 1 of 4 possible categories (blue animal, red animal, blue household object, red household object), this image is immediately followed by a probe which the participant must respond to as rapidly as possible to maximise potential monetary reward (the magnitude of which is proportional to speed of response). One dimension of the pre-probe category is relevant to the probability of reward (e.g. if colour is the relevant dimension, 90% of probes following a red image would be accompanied by a reward, in contrast to 10% of probes following a blue image), while the other is irrelevant (e.g in the previous case, both animals and household objects would have a 50% chance of being followed by a reward).

Two runs (64 trials each) were performed. Results are obtained for adaptive (relevant) and aberrant (irrelevant) salience, both based on participant reported estimated probabilities on visual analogue scales (SAT explicit salience) and reaction times (SAT implicit salience). SAT explicit adaptive salience represents the extent to which participants report a reward as more likely to follow following the relevant stimuli, compared to irrelevant stimuli. SAT implicit adaptive salience is a measure of how much more quickly a participant reacts to stimuli associated with reward in the relevant dimension. Measures of SAT aberrant salience relate to how much more likely participants rate or respond across the irrelevant dimension. For detailed methods see previously published reports (Roiser et al., 2013, 2009).

SAT implicit aberrant, SAT explicit aberrant, and SAT implicit adaptive scores, and aberrant salience inventory scores were skewed and therefore square root transformed prior to analysis as previously recommended (Roiser et al., 2009).

2.3 Demographic and Behavioural Data Analysis

All statistical analysis was performed using R version 3.3.2. The normality of variables was checked using the Shapiro-Wilks test, and results were square root transformed if skewed (Roiser et al., 2009). The significance of differences between groups for continuous variables was determined using an independent samples t-test. Pearson's $\chi 2$ test was used to test for group differences regarding categorical variables.

2.4 Resting State Functional Magnetic Resonance Imaging

2.4.1 rfMRI: Data Acquisition

Imaging data was acquired using a Philips 3T Intera magnetic resonance imaging system. A ten-minute resting state scan was performed using a T2* weighted transverse echo planar image sequence (TR = 3000 ms, TE = 30 ms, flip angle = 90°, slice thickness = 3.25mm, 2.19mm x 2.19mm in plane resolution, 44 slices, 200 volumes). A T1 structural image was then obtained with a gradient-echo scan (TR=9.7ms, TE=4.6ms, flip angle=90°, slice thickness=1.20 mm, 0.94 x 0.94 mm in plane resolution, 150 slices).

2.4.2 rfMRI: Seed Definition

Striatal seeds consisting of bilateral 3.5mm radius spheres were placed in the inferior ventral striatum, superior ventral striatum, dorsal caudate, dorsal rostral putamen, dorsal caudal putamen, and ventral rostral putamen. These predefined seeds were initially reported by Di Martino et al,(Di Martino et al., 2008) and have

been repeatedly used in investigations of striatal connectivity (DelDonno et al., 2017; Fornito et al., 2013; Gabbay et al., 2013; Sarpal et al., 2016).

2.4.3 rfMRI: Preprocessing

fMRI data was analysed using the CONN toolbox (version 17) implemented in SPM12 (Whitfield-Gabrieli and Nieto-Castanon, 2012). A standard preprocessing pipeline was used consisting of slice timing correction, realignment, and normalisation to MNI space based on segmentation parameters derived from segmentation of the T1 structural image. Images were smoothed with a Gaussian kernel of 6 mm full-width-half-maximum.

The Artifact Detection Tools (ART) toolbox (www.nitrc.org/projects/artifact_detect) was used to account for motion and artefact detection using anatomical component based correction (aCompCor) of temporal confounds relating to head movement and physiological noise. This method models noise effects at a voxel level based on estimates derived from principal components of noise regions of interest (white matter and CSF, eroded by one voxel to minimise partial volume effects), and then removes these from the BOLD timeseries using linear regression, global signal regression is not performed. Six residual head motion parameters and their first order temporal derivatives were also entered as regressors into the first level model, as was an effect accounting for magnetisation stabilisation and its first order derivative. Artifact/outlier scans (average intensity deviated more than 5 standard deviations from the mean intensity in the session, or composite head movement exceeding 0.9 mm from the previous image) were also regressed out. Preprocessed data were temporally bandpass filtered (0.008-0.09 Hz)

2.4.4 rfMRI: Connectivity analysis

Voxel wise connectivity maps for each participant were then derived by computing Pearson correlations between the signal average over each seed region, and the

signal at each voxel over the entire brain. These were then converted to normally distributed Fisher's Z maps to allow second level general linear model analyses. At the second level, connectivity maps between exposed and unexposed groups were contrasted with each other for each seed (left and right hemisphere seeds were entered into the same model, so six group level comparisons were performed in total). Clusters were considered statistically significant if they passed height thresholds of $P<0.001$ and cluster-level thresholds of $P<0.05$ FWE-corrected.

2.5 rfMRI Relationship with Salience Measures

Fisher transformed correlation coefficients were extracted from significant clusters and averaged (weighted by cluster size) for each of the seeds showing statistically significant results. Pearson correlation coefficients were then calculated between these connectivity values and participant salience scores, with Spearman correlations also performed to ensure results were not outlier driven. We next performed an exploratory analysis investigating whether the difference between exposed and unexposed groups' salience scores might be mediated by altered corticostriatal connectivity. Where we had observed a significant bivariate relationship between corticostriatal connectivity and salience scores we tested a mediation model using the R package 'mediation' using quasi-Bayesian MonteCarlo simulation (10,000 simulations) to test for significance (Tingley, D., Yamamoto, T., Hirose, K., Keele, L., & Imai, 2009).

Results

3.1 Participant Demographics

22 unexposed and 20 exposed participants were recruited; demographics are displayed in Table 1. No significant differences existed between groups for age or sex. As expected, measures of childhood trauma, population density and migration and were significantly different between the exposed and unexposed group.

	Unexposed (n=22)	**Exposed(n=20)**	**P-value**
Age (years), mean (SD)	26.3 (±6.5)	27.2 (±7.1)	0.67[b]
Sex *n* (%female)	11 (50.0%)	11 (55.0%)	0.99[c]
Aberrant Salience Inventory[a]	1.8 (±1.6)	2.7 (±1.3)	0.044[b]
Childhood Trauma Questionnaire	29.2 (±5.4)	36.0 (±9.0)	0.005[b]
Population Density (persons per hectare)	21.2 (±17.5)	81.2 (±32.5)	< 0.001[b]
% 1st Gen. Migrant	0 (0.0%)	7 (35.0%)	0.009[c]
% 2nd Gen. Migrant (both parents)	0 (0.0%)	9 (45.0%)	0.002[c]
% 2nd Gen. Migrant (one parent)	3 (13.6%)	3 (15.0%)	1.00[c]
Mean Motion	0.2 (±0.1)	0.1 (±0.1)	0.76[b]
Maximum Motion	1.4 (±2.3)	1.7 (±2.3)	0.69[b]
Valid Volumes	193.1 (±14.2)	195.6 (±7.3)	0.49[b]

[a] Square root transformed
[b] Independent samples t-test
[c] Chi-square test

Table 1: Demographic details of participants, salience scores, and MRI movement.

3.2 Salience Measures

The exposed group displayed significantly reduced SAT explicit adaptive salience (Cohen's d=0.69, p=0.03, df=40), and increased aberrant salience inventory scores (d=0.65, p=0.04, df=40) compared to the unexposed group (see figure 2). There were no other significant differences between groups on SAT measures. Within the whole group, SAT explicit aberrant and SAT explicit adaptive salience were negatively correlated (rp=-0.44, p=0.004, df=40). There were no other statistically significant correlations between items of the SAT, nor between the SAT items and the ASI.

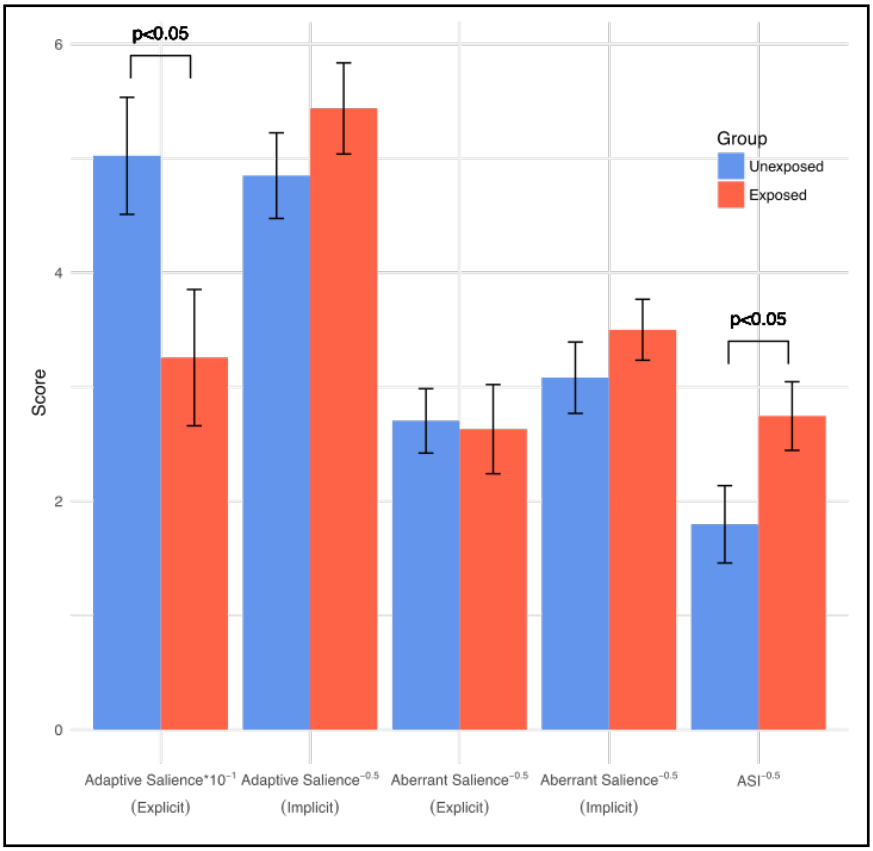
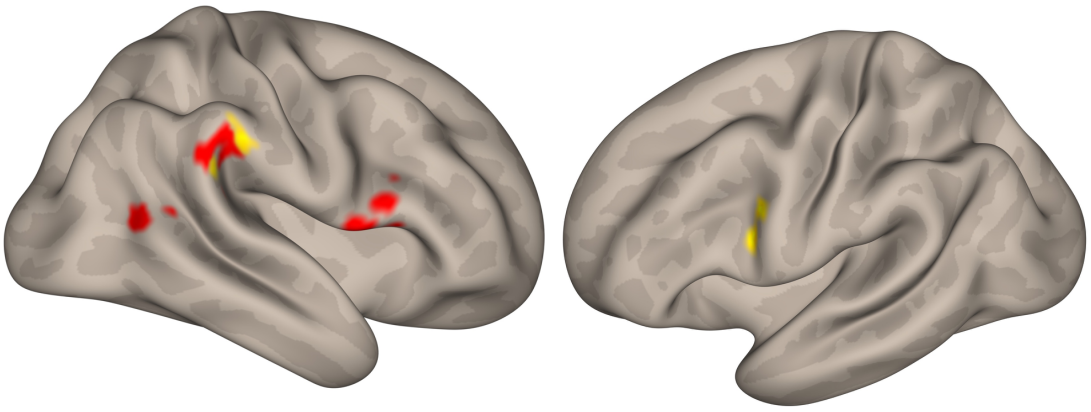


Figure 2. Salience attribution test, and aberrant salience inventory scores in exposed and unexposed individuals.
Error bars (+/- 1SE) ASI – Aberrant Salience Inventory.

3.3 rfMRI Data

There were no differences between groups in terms of motion during the scan (see table 1). Compared to the unexposed group, the exposed group showed increased connectivity between the inferior ventral striatum and three clusters (see figure 3). These clusters were centred on the right supramarginal gyrus, insular operculum and middle temporal gyrus, and the largest would survive Bonferroni correction for the six seeds examined (0.05/6=p<0.0083). The dorsocaudal putamen also displayed increased connectivity in the exposed group, with significant clusters centred on the right supramarginal gyrus and left insular operculum. The largest of the dorsocaudal putamen clusters would also pass Bonferroni correction. No significant clusters were identified for the other seeds. No seeds displayed increased connectivity in the unexposed group.



Seed	MNI coordinates	Cluster size	P*
Inferior Ventral Striatum	+58 +14 +22	219	0.00076
Inferior Ventral Striatum	+64 -42 +28	138	0.013
Inferior Ventral Striatum	+48 -56 +6	126	0.020
Dorsocaudal Putamen	+66 -26 +32	201	0.0017
Dorsocaudal Putamen	-44 -06 +30	124	0.016

*FWE corrected after initial p<0.001 cluster defining threshold

Figure 3. Areas of increased corticostriatal connectivity in the exposed compared to unexposed group.
Red clusters relate to the seed in the inferior ventral striatum, and yellow represents the dorsocaudal putamen seed.

3.4 rfMRI Relationship with Salience Measures

To investigate the potential behavioural relevance of the imaging findings, we averaged the connectivity z values within the significant clusters for each of the seeds (weighted by cluster size) to give a value for both average inferior ventral striatum connectivity, and dorsocaudal putamen connectivity. We then investigated correlations between these two summary connectivity measures and the behavioural measures.

Within the whole group inferior ventral striatum connectivity negatively correlated with SAT explicit adaptive salience scores (rp=-0.48, p=0.001, df=40, see figure 4A) and this was also present within the exposed group alone (rp=-.52, p=0.018, df=18). Inferior ventral striatum connectivity and SAT explicit aberrant salience scores were negatively correlated within the exposed group (rp=0.523, p=0.016, df=18, see Figure 4B), but not across the whole group. Inferior ventral striatum connectivity also positively correlated with Aberrant Salience Inventory scores across the whole group (rp=0.42, p=0.0056, df=40, see Figure 4C). All these correlations remained significant when using Spearman's coefficient, indicating statistical significance was not outlier driven.

Dorsocaudal putamen connectivity also showed a negative correlation with SAT explicit adaptive salience scores (rp=-0.36, p=0.020, df=40) across the whole group, and a positive correlation with SAT explicit aberrant salience only in the exposed group (rp=0.50, p=0.025, df=18). When using Spearman's coefficient the whole group correlation remained significant but the exposed group did not (rs=0.43, p=0.059).

We next performed an exploratory mediation analysis to investigate whether the relationship between risk factor exposure (i.e. the binary variable of group) and

salience scores might be mediated by altered corticostriatal connectivity. In the case of the inferior ventral striatum this showed a significant mediation effect for explicit adaptive SAT scores (indirect path estimate ß=-17.8, 95% CI=-34.5, -3.2, p=0.02; direct path estimate ß 0.14, CI=-20.01, 20.87, p=0.99, see figure 4D), and aberrant salience inventory scores (indirect path estimate ß=4.5, 95% CI=0.35, 9.26, p=0.03; direct path estimate ß -0.885, CI=-6.65, 5.02, p=0.77). The indirect path estimate between dorsocaudal putamen connectivity and explicit adaptive SAT scores was not significant (ß=-9.6, 95% CI =-27.9, 7.4, p=0.28).

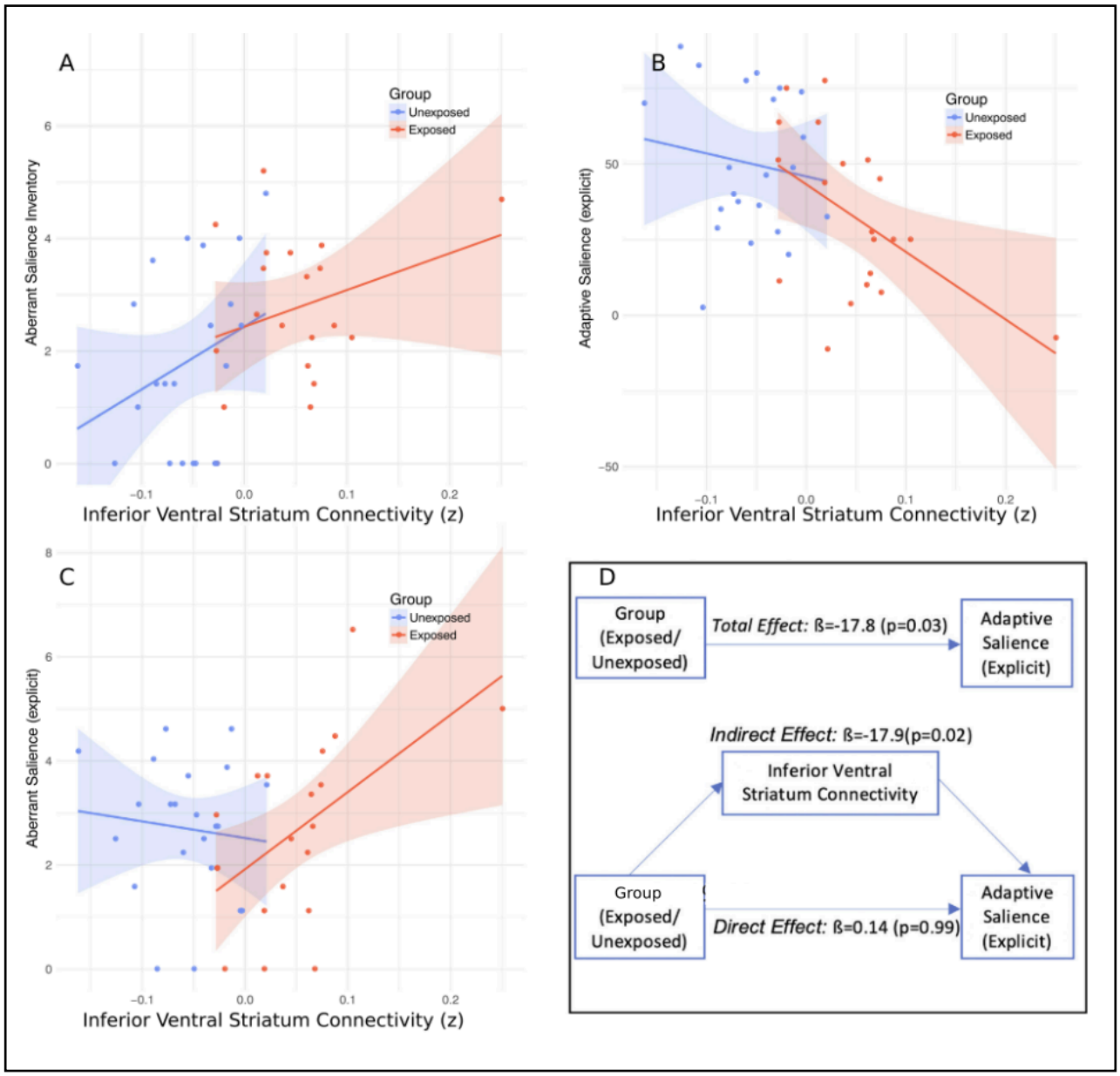


Figure 4. Relationship between salience scores and inferior ventral striatum connectivity

(A) Inferior ventral striatum connectivity correlates with explicit adaptive SAT scores in both the whole sample (r_p=-0.48, p=0.001) and exposed individuals (rp=-0.52, p=0.001)

(B) Inferior ventral striatum connectivity correlates with explicit aberrant SAT scores in exposed individuals (r_p=0.523, p=0.016)

(C) Inferior ventral striatum connectivity correlates with Aberrant Salience Inventory Scores in the whole sample (r_p =0.42, p=0.0056)

(D) Mediation analysis – the relationship between risk factor exposure (i.e. whether participants in the exposed or unexposed group) and reduced SAT adaptive salience appears to be mediated by altered inferior ventral striatum connectivity.

Discussion

In the current study, we demonstrated reduced adaptive salience in individuals that had been exposed to chronic psychosocial stressors, and found that this was related to increased connectivity between striatal seeds and cortical regions involved in salience processing. We also found increased scores on the aberrant salience inventory in the exposed group, but contrary to our initial hypotheses did not detect any between group differences on the aberrant or implicit measures of the SAT.

The exposed group demonstrated reduced SAT explicit adaptive salience, which represents a relative impairment in the learning of stimulus-reward associations. Dopamine neurons projecting to the ventral striatum play a central role in this process (Schlagenhauf et al., 2013; Schultz et al., 1997), and impaired dopaminergic reward signalling secondary to chronic stress has been put forward as one of the neurochemical alterations contributing to affective (Cabib and Puglisi-Allegra, 2012; Chen et al., 2015) and psychotic disorders (Howes et al., 2017). Reduced adaptive salience has been previously demonstrated in psychosis, and it was initially suggested that this primarily existed as a result of treatment with dopamine antagonists (Roiser et al., 2009). However, studies in un-medicated individuals at high risk of the disorder have displayed a similar pattern (Roiser et al., 2013; Schmidt et al., 2016), and some models suggest that although psychosis is associated with increased aberrant dopamine signalling (McCutcheon et al., 2018a), this may be accompanied by reductions in adaptive signalling (Maia and Frank, 2017). The exposed group also demonstrated increased scores on the ASI but not on any of the SAT measures of aberrant salience. This is similar to several studies in clinical populations, where it appears that the measure of SAT explicit adaptive salience is the most sensitive to group differences (Abboud et al., 2016; Katthagen et al., 2016; Pankow et al., 2016; Roiser et al., 2009). In terms of the magnitude of

effect, the effect size observed in the current study for the measure of explicit adaptive salience (d=0.69), was smaller than what has been observed in schizophrenia (d=1.08 (Roiser et al., 2009)), but larger than what was reported in a recent study of individuals at clinical high risk of schizophrenia (d=0.25, (Roiser et al., 2013)). It may be that alterations in adaptive salience processing occur more readily in the face of chronic stress, while increases in aberrant salience scores are only observed in established illness. The lack of group differences in implicit measures is something that has also been observed in some patient cohorts, and the correct interpretation of these measures remains unclear(Abboud et al., 2016; Neumann and Linscott, 2018; Roiser et al., 2013).

The exposed group also displayed increased functional connectivity between the ventral striatum and several cortical regions. A number of these clusters overlap with cortical areas that make up the cingulo-opercular or salience network (Gordon et al., 2016). This network is involved in the detection of relevant stimuli and the coordination of switching to appropriate brain states (Uddin, 2015), and shows an association with striatal dopamine function (McCutcheon et al., 2018c). Increased connectivity of the ventral striatum has also been observed in individuals with schizophrenia (Fornito et al., 2013), those at increased risk of the disorder (Dandash et al., 2014; Fornito et al., 2013), and those specifically affected by hallucinations (Rolland et al., 2015). In contrast to the current results, two of these studies simultaneously observed a pattern of reduced connectivity with the dorsal striatum (Dandash et al., 2014; Fornito et al., 2013). The pattern of connectivity alterations observed in the exposed group, therefore overlapped to an extent with what has been previously observed in schizophrenia, but also showed significant differences.

The environmental exposures, neural circuits, and cognitive mechanisms we studied have all been implicated in schizophrenia, but have transdiagnostic relevance, and

as such may be best interpreted as mechanisms of general relevance to psychopathology rather than being disorder specific (Insel et al., 2010). Increased functional connectivity of the ventral striatum in has also been linked to an increased risk of subsequently developing depression (Pan et al., 2017), although findings in those with the established disorder have been inconsistent (Furman et al., 2011; Gabbay et al., 2013). This may be secondary to pathophysiological heterogeneity, illustrated by the fact that increased ventral striatum connectivity has been specifically observed in a high anxiety subgroup (Drysdale et al., 2016), while reduced connectivity has been observed in a high inflammation subgroup (Felger et al., 2016).

Recent work has also found that both early life adversity (Hanson et al., 2018), and economic disadvantage (Marshall et al., 2018) are associated with increased functional connectivity of the ventral striatum; although earlier work demonstrated a reduction in ventral striatum connectivity in those raised in households of lower parental education (Gianaros et al., 2011). Another study reported reduced functional connectivity of the caudate and putamen during an error monitoring task but did not use a ventral seed (Hart et al., 2018). Studies of urbanicity and migration have not directly examined corticostriatal connectivity, although mesolimbic signalling has been shown to relate to urban living (Kramer et al., 2017), and altered activation of the ventral striatum has been demonstrated in migrant individuals (Akdeniz et al., 2014).

We found that differences in ventral striatum connectivity mediated the association between chronic social stress and both reduced adaptive salience scores, and increased aberrant salience inventory scores. While the whole striatum appears to play a role in salience processing (Ilango et al., 2014; Oyama et al., 2015), it is the ventral striatum that has been principally implicated in studies using the SAT (Boehme et al., 2015; Roiser et al., 2010), and it is the ventral striatum that shows

alterations in dopamine function in studies of early life stress(Oswald et al., 2014; Pruessner et al., 2004). It is, however, the associative striatum that displays the most marked dopaminergic dysfunction in schizophrenia (McCutcheon et al., 2018a), and the precise mechanism through which corticostriatal connectivity contributes to salience attribution is yet to be fully elucidated, although it is likely that dopaminergic mechanisms contribute (Bell et al., 2015; Boehme et al., 2015; Nagy et al., 2012; Roiser et al., 2010).

The study of risk factor exposure in individuals free of psychiatric illness means that the effect of exposure is not obscured by the presence of disease; however, a limitation is that it also raises the possibility that observed differences are markers of resilience as opposed to sequelae of exposure.

Psychosocial stress is a multidimensional construct and as a result we studied multiple stressors. The threshold used for defining groups in the case of migration was based on the finding that both first and second generation immigrants have an increased risk of psychosis,(Bourque et al., 2011), childhood adversity thresholds were based on previous studies (Kraan et al., 2017; Philip et al., 2014), while our cutoff for urbanicity was based was on census data for population densities in London suburbs. It may have been beneficial to specify even lower cutoffs regarding population density for the unexposed group, although this may have impeded recruitment. Our approach aimed to maximise the distance between groups in terms of exposure, however the resulting collinearity of risk factor exposures means that we were unable to determine the extent to which each individual exposure drives the observed group differences. While a limitation, this is typically an issue in single exposure studies as well (albeit a less explicit one), given that these do not tend to measure other potential risk factors, and the fact that these stressors show a tendency to cluster (Hjern et al., 2004; Morgan et al., 2007; Wicks et al., 2005).

A further potential limitation is the fact that apart from the inverse correlation between the two explicit SAT measures no significant association was observed between the other salience measures. This has been previously studied, and while this may be a result of the various measures capturing different aspects of salience processing it also suggests caution may be required when interpreting the meaning of these measures (Neumann and Linscott, 2018). In addition, the results of the mediation analysis only just reached statistical significance, and should be viewed with caution given the relatively low sample size for this form of analysis.

Future research has the potential to address several of the study's limitations. Longitudinal follow up can clarify whether observed behavioural and neurobiological differences relate primarily to resilience or risk. Studies in patient populations, also have the potential to determine the pathophysiological relevance of our findings. Future studies might consider a factorial design, which would enable testing as to specific effects of individual exposures, and whether additive and synergistic effects exist. Even when considering only three risk factors, however, eight possible combinations exists and a large sample would therefore be required. Alternatively using a continuous exposure score would be a powerful approach, although while recent scores have been proposed these remain to be validated (Padmanabhan et al., 2017; Vassos et al., 2018). Studies using positron emission tomography may allow for the investigation of whether the connectivity and behavioural differences observed involve dopaminergic alterations.

In conclusion, we found evidence that exposure to chronic psychosocial stressors was associated with alterations in salience processing and corticostriatal connectivity. Longitudinal studies may help probe the implications that this holds for the development of psychiatric disorders. In addition, future studies using

multimodal methodologies are necessary to further understand the relationship between dopaminergic systems and functional connectivity.

Contributors

RAM, MAPB, TD & ODH participated in the design of the study. RAM collected the data used in the study. RAM, MM and ODH were involved in the analysis of data. RAM and ODH wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

O.H. has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organised by Astra-Zeneca, Autifony, BMS, Eli Lilly, Heptares, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. Neither Dr Howes or his family have been employed by or have holdings/ a financial stake in any biomedical company. M.M. has consulted for Cambridge Cognition, Lundbeck and Forum Pharmaceuticals in the past 3 years. He has also received research funding from Takeda, Eli Lilly and Roche. The other authors declare no competing financial interests.

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6. Mesolimbic Dopamine Function is Related to Salience Network Connectivity: An Integrative PET and MR Study

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Abstract

Background

A wide range of neuropsychiatric disorders from schizophrenia to drug addiction involve abnormalities in both the mesolimbic dopamine system and the cortical salience network. Both systems play a key role in the detection of behaviourally relevant environmental stimuli. Although anatomical overlap exists, the functional relationship between these systems remains unknown. Preclinical research has suggested the firing of mesolimbic dopamine neurons may activate nodes of the salience network, but in vivo human research is required given the species-specific nature of this network.

Methods

We employed positron emission tomography to measure both dopamine release capacity (using [^{11}C]-(+)-PHNO, n =23), and dopamine synthesis capacity (using 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine, n =21) within the ventral striatum. Resting-state functional magnetic resonance imaging was also undertaken in the same individuals to investigate salience network functional connectivity. A graph theoretical approach was used to characterise the relationship between dopamine measures and network connectivity.

Results

Dopamine synthesis capacity was associated with greater salience network connectivity, and this relationship was particularly apparent for brain regions that act as information processing hubs. In contrast, dopamine release capacity was associated with weaker salience network connectivity. There was no relationship between dopamine measures and visual and sensorimotor networks, indicating specificity of the findings.

Conclusions

Our findings demonstrate a close relationship between the salience network and mesolimbic dopamine system, and are relevant to neuropsychiatric illnesses in which aberrant functioning of both systems has been observed.

Introduction

Resting state functional magnetic resonance imaging (rfMRI) has demonstrated that activity within networks of brain regions is temporally correlated even in the absence of explicit external demands(1), and furthermore that these networks underlie human cognition and behaviour(2,3). The salience network, also referred to as the cingulo-opercular network, is centred around the anterior insula and dorsal anterior cingulate, and in some instances has also been proposed to contain subcortical structures including the limbic (ventral) striatum and substantia nigra(4,5). Recent meta-analyses synthesising structural and functional imaging data have identified this network as uniquely affected across psychiatric disorders(4,5).

The salience network plays a key role in identifying the most relevant internal and external stimuli in order to guide behaviour appropriately(6–11). Connectivity within the salience network is increased by externally-directed demands, which contrasts with the default mode network (centred around the ventromedial prefrontal cortex and the posterior cingulate cortex)(12,13), where connectivity is enhanced during self-generated thought(14,15). The salience network dynamically coordinates the activity of other networks, in particular switching away from the default mode to task positive networks when appropriate, and impaired communication between the default mode and salience network is seen in a range of disorders, including schizophrenia, drug addiction, and cognitive impairment(16–21).

Dopamine neurons also play a role in the identification of behaviourally relevant environmental stimuli. Mesolimbic dopamine neurons (projecting from the ventral tegmental area to the limbic striatum) have been proposed to signal *reward* prediction errors, which signal the discrepancy in the observed and predicted *value* of a stimulus(22). More recent research, however, has shown that these neurons

respond to surprising stimuli even in the absence of any change in value, suggesting that their role extends to assigning salience to relevant environmental stimuli in general, not solely on the basis of value(23,24). Dysfunction of this system is also observed in many neuropsychiatric illnesses(25,26).

The need to develop an integrative understanding regarding the roles of the salience network and the mesolimbic dopamine system has been previously stressed(9). Given their overlap in function, it may be hypothesised that mesolimbic dopamine signalling plays a role in the modulation of the salience network. Recently, chemogenetic, optogenetic, and electrical stimulation of mesolimbic dopamine neurons in rodent models has been shown to activate salience network nodes, including regions not directly innervated by the ventral tegmental area(27–30). While cross species similarities exist in the organisation of cortical networks, there are also marked differences. Longer distance connections in particular are proportionally much weaker in primates, potentially contributing to an increased vulnerability to 'disconnection syndromes' such as schizophrenia(31). As a result, in vivo human research is required for a comprehensive understanding of the relationship between network connectivity and neurochemical signalling. Human studies have demonstrated effects of pharmacological dopaminergic challenges on salience network connectivity, suggesting dopamine might regulate the salience network in humans, but, crucially, these studies are limited in their explanatory potential because of the non-physiological and anatomically non-specific effects of the intervention(32–34). Thus, it remains unclear if mesolimbic dopaminergic signalling is linked to the salience network in humans.

To address this, we employed positron emission tomography (PET) to measure both dopamine synthesis capacity and dopamine release capacity, and rfMRI to evaluate salience and default mode networks at rest in the same subjects. Based on recent preclinical findings that stimulation of dopamine neurons projecting to the limbic

striatum activates regions of the salience network(27–29), our primary hypothesis was that individuals with greater striatal dopamine synthesis and release capacity would show greater connectivity within the salience network, and, due to the reciprocal relationship between salience and default mode networks, weaker connectivity within the default mode network(27).

In addition, we identified within these networks, regions that played the most important role in information processing ('hub nodes'). Hubs support the rapid integration of information across a complex system, and as such can be considered an optimal target via which a network input may efficiently maximise its influence in a coordinated fashion(35,36). We therefore hypothesised that there would not be a uniform association between dopamine function and connectivity, but rather that hub nodes would show the strongest association with dopamine function.

Given the preclinical emphasis on the mesolimbic dopamine projection, we focused on dopamine measures within the limbic striatum. However, we also explored the relationship between network connectivity and dopamine function in the associative and sensorimotor (dorsal) striatum. In addition, to provide a control condition, we investigated the relationship between striatal dopamine function and the visual and sensorimotor networks (networks not directly involved in salience processing), where we did not expect a relationship to be present.

Methods

The experimental approach is summarised in Figures 1 and 2. PET was used to investigate two different aspects of dopaminergic functioning. In experiment 1 we measured dopamine *synthesis* capacity, while in experiment 2 we measured dopamine *release* capacity. rfMRI was used to investigate salience and default mode network connectivity. The relationship between salience/default mode connectivity, and dopamine function was then investigated using a graph theoretical approach in which brain regions are represented as nodes, and functional connections between these regions are represented as edges linking these nodes.

We first investigated whether network connectivity was associated with measures of dopamine function, and identified specific nodes that were associated with dopamine function. We then separately classified nodes as information processing hubs solely based upon their pattern of rfMRI connectivity, and determined whether dopamine-associated nodes overlapped significantly with these hub nodes.

In addition, the visual and sensorimotor networks were examined as control networks as they are not directly involved in salience processing, and show a lack of activation in preclinical studies of mesolimbic dopamine effects(27–29). Further details are given below and in the supplementary information.

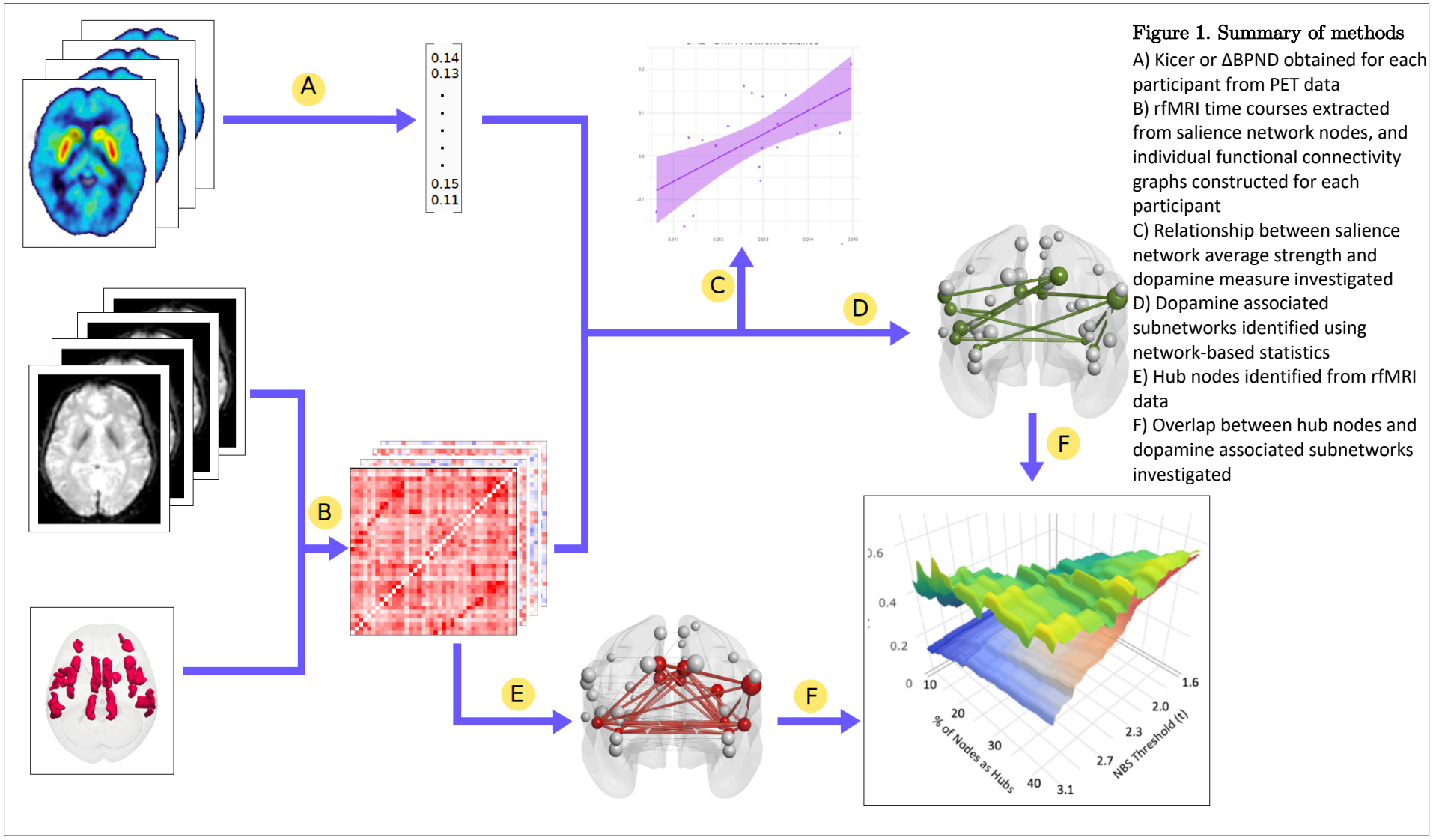


Figure 1. Summary of methods

A) Kicer or ΔBPND obtained for each participant from PET data

B) rfMRI time courses extracted from salience network nodes, and individual functional connectivity graphs constructed for each participant

C) Relationship between salience network average strength and dopamine measure investigated

D) Dopamine associated subnetworks identified using network-based statistics

E) Hub nodes identified from rfMRI data

F) Overlap between hub nodes and dopamine associated subnetworks investigated

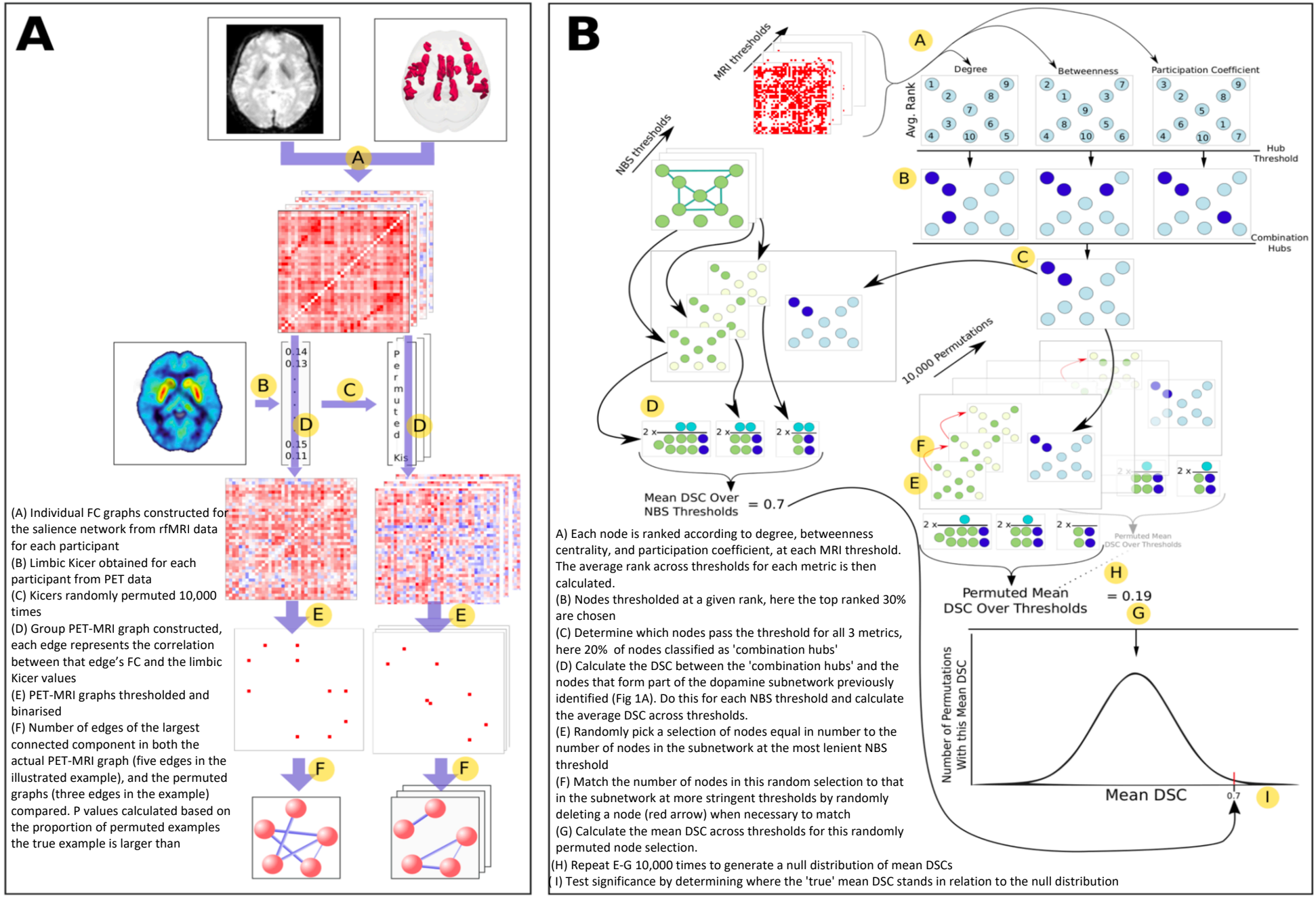


Figure 2. The network based statistic and identifying hub overlap

A) Identifying dopamine associated nodes using the network-based statistic

B) Identifying overlap between dopamine associated nodes and hub nodes

FC – Functional Connectivity, DSC – Dice Similarity Coefficient, NBS – Network Based statistic

Experiment 1: Dopamine synthesis Capacity (^{18}F-DOPA PET)

Participants received a PET scan with the ligand 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (^{18}F-DOPA). ^{18}F-DOPA PET measures the rate constant (K_i^{cer}) for ^{18}F-DOPA uptake, transport into synaptic vesicles, and its conversion into ^{18}F-dopamine, thus providing a measure of dopamine *synthesis* capacity(37).

A region-of-interest analysis was performed to determine the limbic striatum influx constant (K_i^{cer} [1/min])(38). We also determined influx constants for associative and sensorimotor striatum, with these regions defined using the approach outlined by Martinez and colleagues(38).

Participants also received a rfMRI scan on a 3T GE Signa MR scanner.

Experiment 2: Dopamine release capacity (^{11}C-(+)-PHNO PET)

Participants received two PET scans with the D2/3 receptor ligand [^{11}C]-(+)-4-propyl-9-hydroxy-naphthoxazine (^{11}C-(+)-PHNO). A placebo scan gives a measure of baseline D2/3R availability (non-displaceable binding potential, BP_{ND}), while a scan following dexamphetamine administration allows quantification of the change in BP_{ND} due to competition from increased synaptic dopamine concentrations. The percentage reduction in D2/3R availability between placebo and dexamphetamine scans thus provides an index of dopamine *release* capacity. We calculate the percent change in BP_{ND} as follows:

$$\Delta BP_{ND} = 100 \cdot \frac{BP_{ND\,(baseline)} - BP_{ND\,(dexamphetamine)}}{BP_{ND\,(baseline)}}\%$$

Either placebo or 0.5mg/kg dexamphetamine was administered orally 3hrs before ^{11}C-(+)-PHNO administration, so that scan acquisition coincided with the expected

time of peak action(39). ΔBP_{ND} was measured in the same regions as experiment one.

Participants also received a rfMRI scan using a Siemens MAGNETOM Verio 3T scanner.

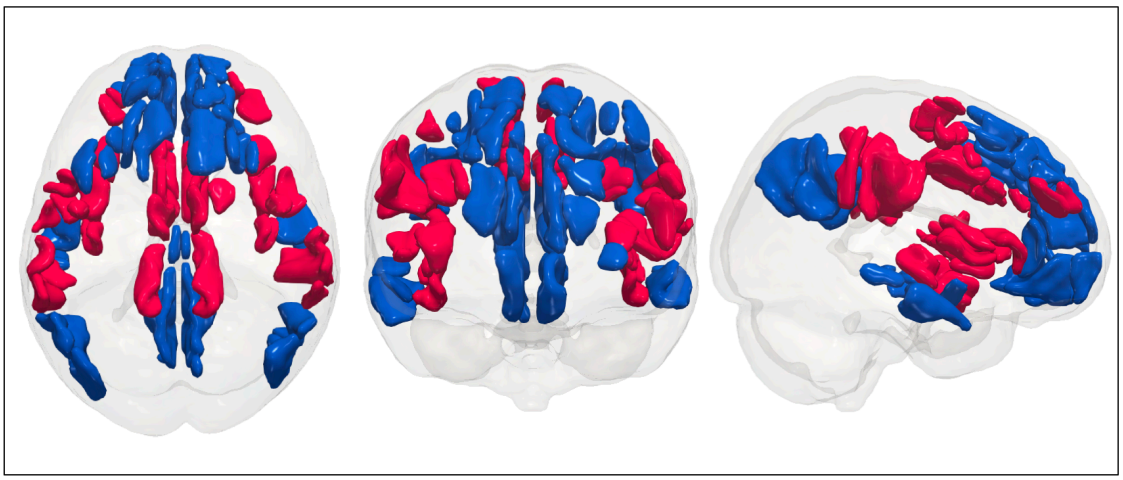
Common methods

Participants

Participants had no previous or current history of psychiatric illness (assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders).

MRI Analysis

Time-series were extracted from N=333 predefined nodes of interests of the Gordon cortical atlas. The salience and default mode network nodes of the Gordon atlas are displayed in eFigure 1. For each participant, a graph representing a functional connectivity network was constructed, each edge representing the level of functional connectivity between a pair of nodes. In order to demonstrate robustness of our findings, we also replicated all analyses using two alternative atlases - the Power(40), and CONN network atlases(41). Furthermore, in addition to using the a priori defined network labels for each node (i.e. salience, default mode etc.), we also ran a whole brain community detection algorithm for each atlas(42), to generate definitions of the salience and default mode networks based on the connectivity patterns present in the current datasets, and repeated our analyses using these data driven node assignments.



eFigure1: Salience and default mode networks of the Gordon atlas

Salience (red) and default mode (blue) network nodes of the Gordon cortical atlas

Network Strength and Dopamine Function

For each participant and each network, average network strength was defined as the mean z-transformed Pearson's correlation coefficient between all network nodes (i.e. mean edge strength)(43). We first calculated Pearson correlation coefficients between network average strength, and the PET measures of dopamine function. We then tested whether the correlation between network strength and dopamine function was significantly different between default mode and salience networks using the method described by Meng et al. as implemented in the *cocor (1.1-3)* package for R 3.3.2(44,45). We also investigated the correlation between dopamine measures and salience-default mode 'balance' (salience network average strength minus default mode network average strength).

Identifying Dopamine-Associated Nodes

In order to identify whether specific nodes show a significant relationship with limbic dopamine synthesis capacity we used the network-based statistic to investigate salience, default mode, sensorimotor, and visual networks separately (see Figure 2A and supplementary methods)(46). Within each network, we identified sub-networks showing a significant relationship with dopamine function and term these 'dopamine-associated subnetworks', and the nodes within these networks 'dopamine-associated nodes'. In addition to examining intra-network connectivity we used the same approach to examine salience and default mode inter-network connectivity. To ensure robustness of the results, this approach was undertaken across a range of network-based statistic thresholds (100 thresholds, t=1.3-3.1, equivalent to p=0.2-0.005 for n=23), where weaker thresholds will capture subnetworks showing a widespread diffuse relationship with dopamine function, and more stringent thresholds identify smaller clusters showing the strongest relationship.

Identifying Network Hubs

Based on the patterns of resting state connectivity within the salience and default mode network we then calculated several graph metrics to identify network hubs. We calculated node degree(47), betweenness centrality(47), and participation coefficient(48). We termed a node that ranked highly on all three metrics a *combination hub* (Figure 2B steps A-C), highlighting its importance as an all-round information processing node. By varying the stringency of criteria used to defined nodes as hubs we defined sets of combination hubs comprising between 10 and 40% of the total number of nodes.

Identifying Overlap Between Dopamine-Associated Nodes and Network Hubs

We next asked whether dopamine-associated nodes were statistically more likely to be combination hubs. We quantified the overlap of dopamine-associated nodes and combination hubs using the Dice Similarity Coefficient (where e.g. A is the set of dopamine-associated nodes and B is the set of combination hub nodes)(49,50):

$$Dice\ Similarity\ Coefficient = \frac{2|A \cap B|}{|A| + |B|}$$

The Dice Coefficient was calculated for each of the 100 NBS thresholds (t=1.3-3.1) and then averaged to give a single score (Figure 2B part D). Permutation testing was used to test whether this overlap score was statistically significant. This procedure was then repeated for each of the combination hub thresholds (10-40%), thereby giving a p-value for each hub threshold.

We also investigated whether there was a significant overlap between ^{18}F-DOPA and ^{11}C-PHNO dopamine-associated nodes.

Results

Participants

Twenty-one participants took part in experiment 1, the ^{18}F-DOPA study (mean(SD) age=23.5 years (3.36); 67 % male). Twenty-three participants took part in experiment 2, the ^{11}C-(+)-PHNO study (age =24.4 years (4.5); 57 % male).

Network Strength and Dopamine Function

Experiment 1: Dopamine synthesis capacity (^{18}F-DOPA)

The correlations between edge strength and limbic dopamine synthesis capacity are displayed in the lower triangle of figure 3A. Average network strength of the salience network positively correlated with limbic dopamine synthesis capacity (r_p=0.51, p=0.017, figure 3B), and this was also significant for all other parcellations (r_p=0.44-0.62, efigure 3). In contrast, average network strength of the default mode network did not show a significant relationship with limbic dopamine synthesis capacity (r_p=-0.32, p=0.16, figure 3B).

The correlation between dopamine synthesis capacity and salience network average strength was significantly different from that between dopamine synthesis capacity and default mode average network strength (z=-2.7, p=0.008). Furthermore, salience-default mode 'balance' (salience network average strength minus default mode network average strength) correlated with dopamine synthesis capacity (r_p = 0.60, p=0.004, Figure 3B).

When the relationship between salience network average strength and dopamine synthesis capacity in other striatal regions was investigated, the findings were

significant for the associative striatum (r_p=0.46, p=0.034) but not the sensorimotor striatum (r_p=0.43, p=0.053) (eFigure 2). As hypothesised, there was no association between limbic dopamine synthesis capacity and average network strength of either the visual (r_p=0.05, p=0.85) or sensorimotor networks(r_p=0.09, p=0.68).

Experiment 2: Dopamine release capacity (^{11}C-(+)-PHNO)

The correlations between edge strength and limbic dopamine release capacity are displayed in the upper triangle of figure 3A. Contrary to our hypothesis, average network strength of the salience network was *negatively* correlated with limbic dopamine release capacity (xr_p=-0.42, p=0.049, figure 3B), a finding that was also significant for some (Gordon data driven, Power data driven) but not all (Power apriori, CONN) of the alternative parcellations (r_p=-0.24 - -0.52, efigure 3). There was no significant correlation between dopamine release capacity and default mode average network strength (r_p=0.03, p=0.9). The difference between these two correlations was not significant (z=1.43, p=0.15), and salience-default mode 'balance' did not correlate significantly with dopamine release capacity (r_p=-0.29, p=0.18).

As in experiment 1, salience network strength was significantly associated with dopamine release capacity in the associative striatum (r_p=-0.5, p=0.015), but showed no relationship with the sensorimotor striatum (r_p=-0.17, p=0.44) (eFigure 2). Furthermore, as in experiment 1, there were no associations between limbic dopamine release capacity and average network strength in either the visual (r=-0.27, p=0.22) or sensorimotor (r=-0.28, p=0.20) networks. Interestingly, however, in an exploratory analysis dopamine release capacity within the sensorimotor striatum showed a significant relationship with sensorimotor network average strength (r=-0.58, p=0.004).

There was no relationship between rfMRI motion and either network strength or dopamine measures (results in supplementary information).

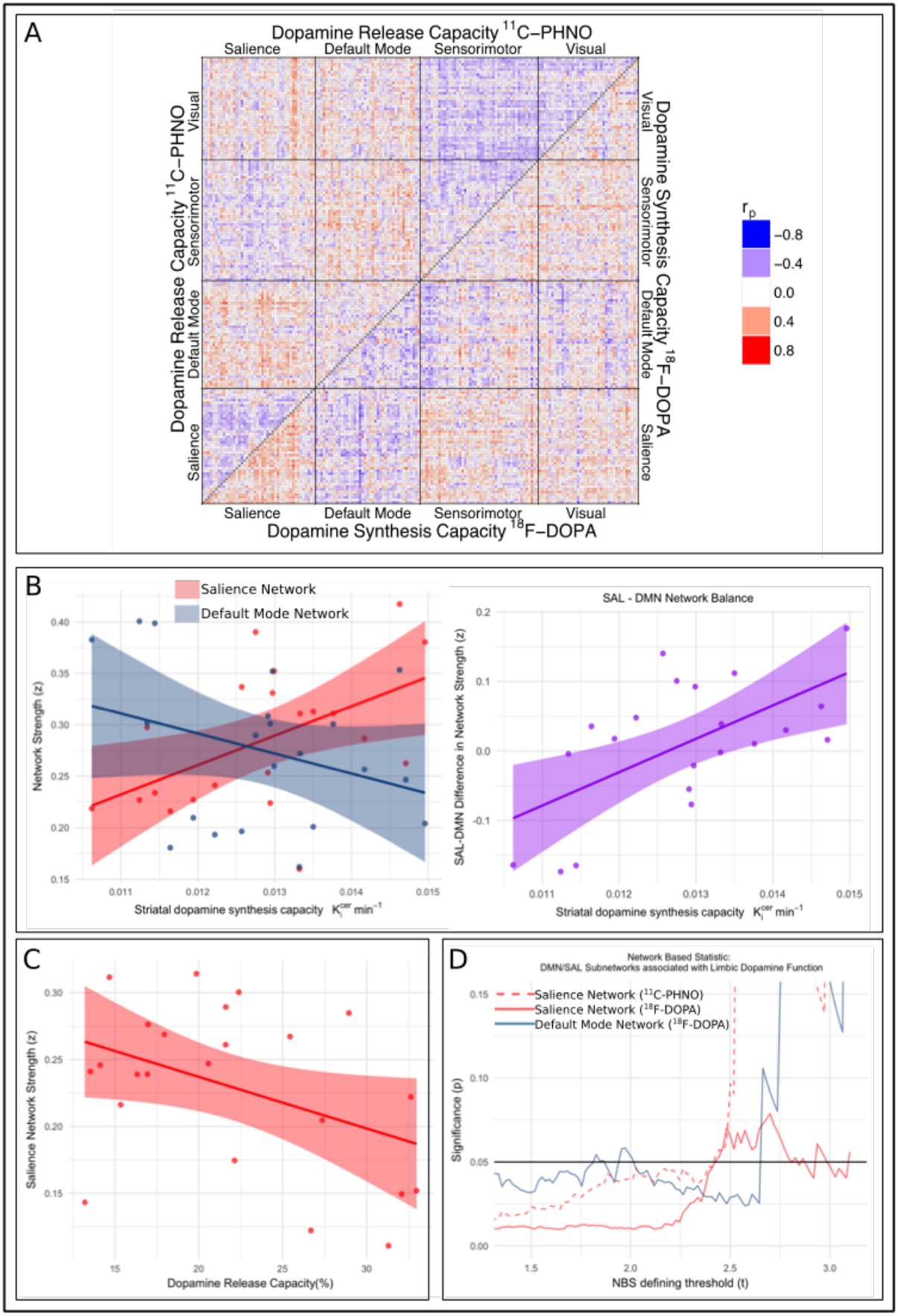
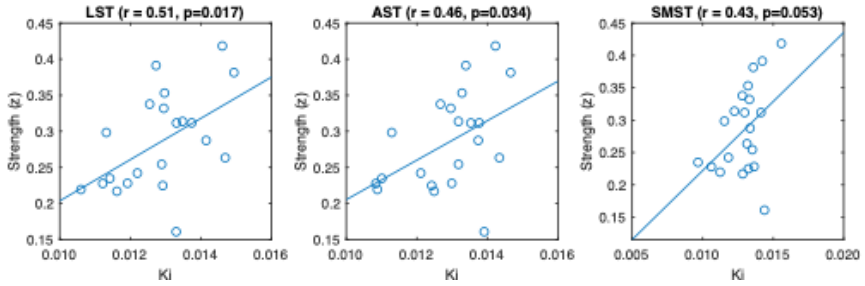


Figure 3: Resting state networks and their relationship with limbic dopamine function

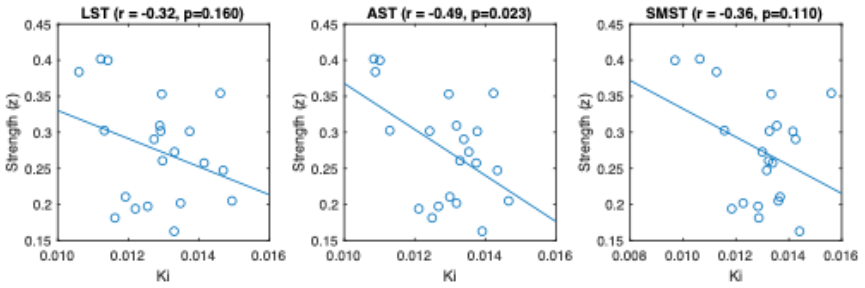
(A) Dopamine associated graphs – each edge represents the correlation between that edge's rfMRI functional connectivity values and limbic dopamine synthesis/release capacity (B) Dopamine synthesis capacity is correlated with salience network strength (N=21, r_p=0.51, p=0.017), did not correlate with DMN strength (r_p=-0.32, p=0.16), and positively correlated with the difference between SAL and DMN strength (r_p = 0.60, p=0.004) (C) Dopamine release capacity negatively correlated with salience network strength (r_p = -0.42, p=0.049) (D)Network-based statistic identifies subnetworks significantly associated with dopamine synthesis /release capacity across a range of thresholds.

eFigure 2: Correlation between average network strength (Gordon parcellation) and dopamine measures in striatal subdivisions

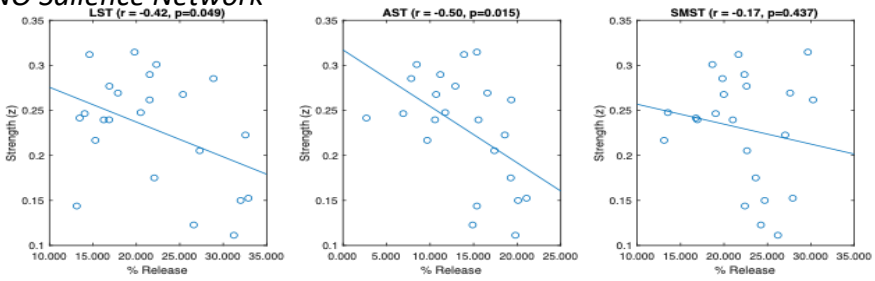
18F-DOPA Salience Network



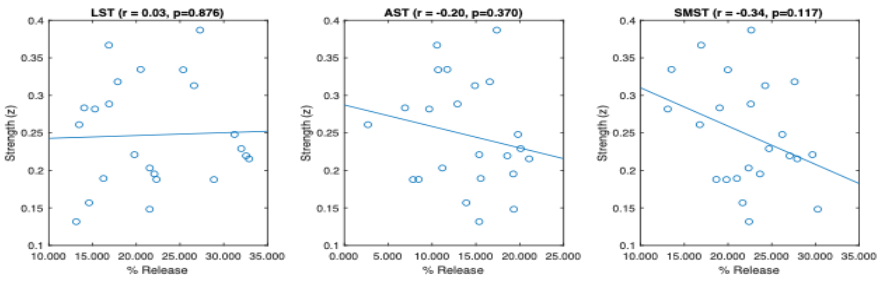
18F-DOPA Default Mode Network



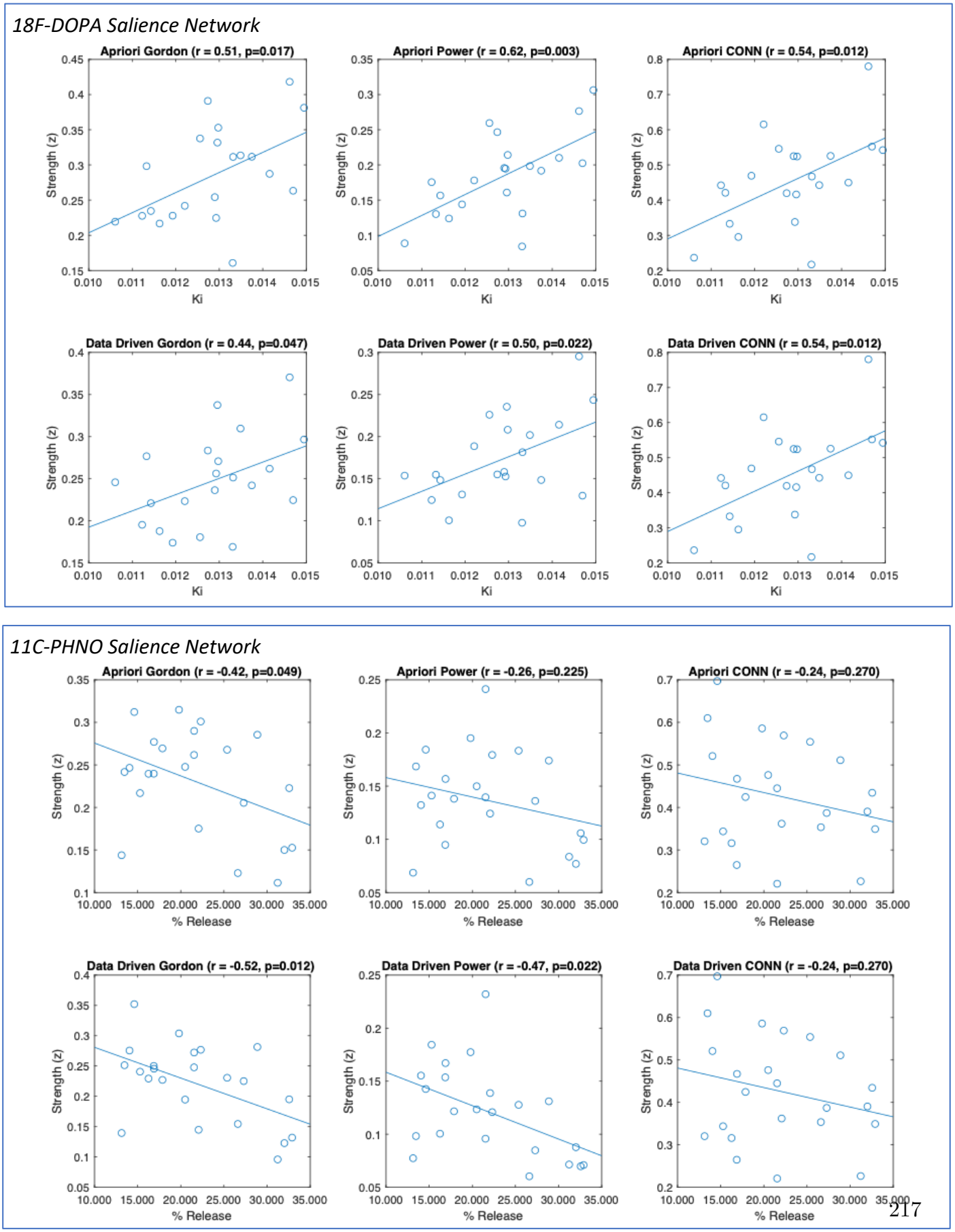
11C-PHNO Salience Network



11C-PHNO Defult Mode Network



eFigure 3: Correlation between limbic dopamine measures and salience network strength for a range of parcellation schemes



Identifying Dopamine-Associated Nodes

Experiment 1: Dopamine synthesis capacity(^{18}F-DOPA)

Using the network-based statistic we identified salience network subnetworks showing a significant positive relationship with limbic dopamine synthesis capacity across a range of thresholds (Figure 3D). In contrast, subnetworks within the default mode network showed a significant negative relationship with dopamine synthesis capacity.

We also used the network-based statistic to examine internetwork connections between default mode and salience networks. At specific thresholds, greater dopamine synthesis capacity was associated with weaker internetwork connectivity (i.e. greater decoupling), although this was not significant across a wide range of thresholds (eFigure 4).

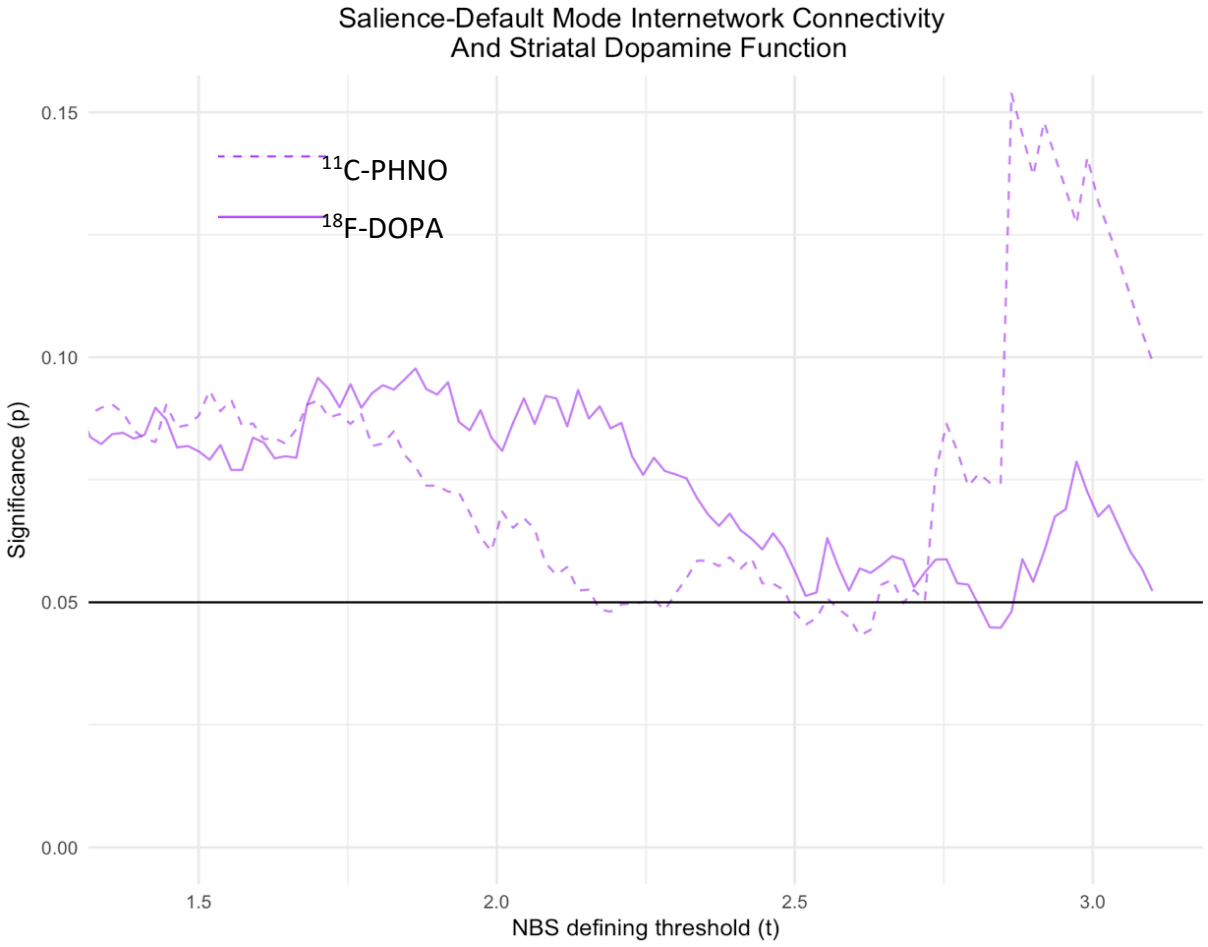
When dopamine synthesis capacity in other striatal subdivisions was examined, the findings were again significant for the associative but not sensorimotor striatum (eFigure 5). The specificity of the findings was again demonstrated by the fact that no dopamine associated subnetworks were identified in either visual ($p>0.29$ for all thresholds) or sensorimotor ($p>0.38$) networks.

Experiment 2: Dopamine release capacity (^{11}C-(+)-PHNO)

We identified subnetworks within the salience network showing a significant negative relationship with limbic dopamine release capacity (Figure 3D). No default mode subnetworks showed a significant association with dopamine release capacity. As in experiment 1 examination of internetwork connections suggested that release capacity was associated with internetwork coupling only at specific thresholds, and in this case greater release capacity was associated with stronger coupling (eFigure 4).

Dopamine release in other regions was examined and similarly to experiment 1, significant results were observed for the salience network with the dopamine measure in the associative striatum but not sensorimotor striatum (eFigure 5). As before we demonstrated specificity of findings in that no visual ($p>0.12$ all thresholds) or sensorimotor subnetworks ($p>0.11$ all thresholds) were associated with limbic dopamine release capacity.

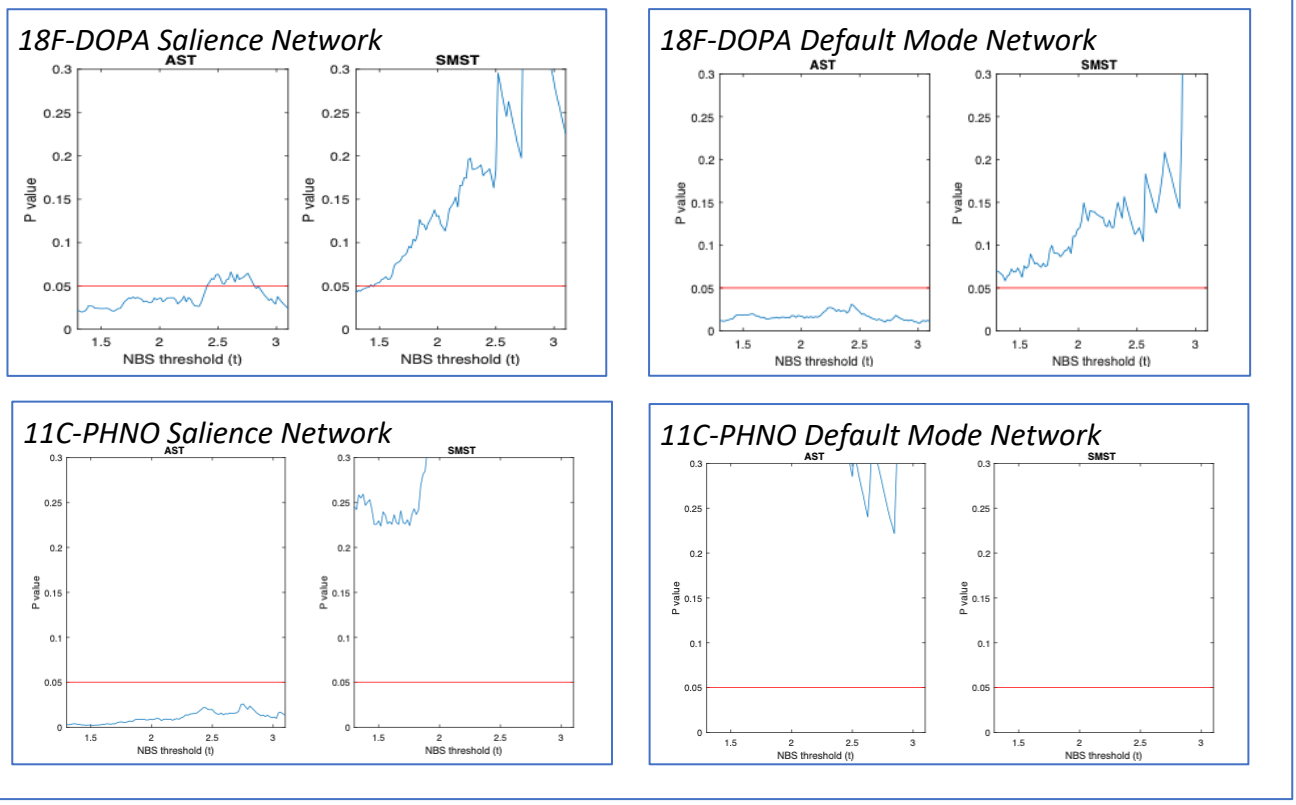
In both experiments these findings were seen in various parcellations and methods of node assignment (eFigure 6).



eFigure 4. Relationship between salience-default internetwork connectivity and limbic dopamine function

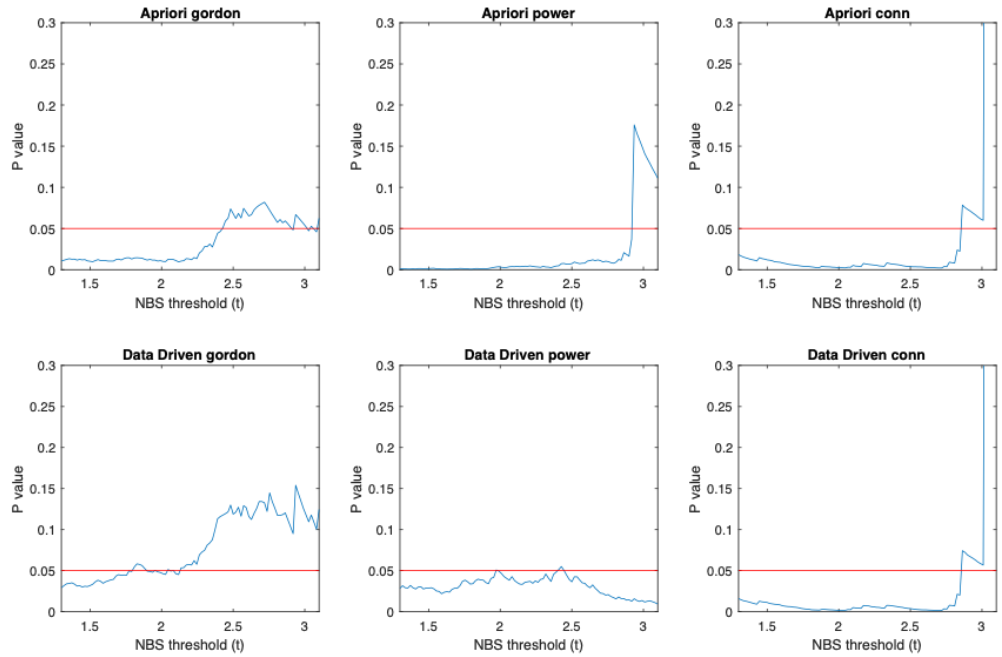
For ^{11}C-PHNO the p-value represents the significance of the relationship between greater BPND and greater internetwork connectivity as calculated using NBS, while for ^{18}F-DOPA this refers to an association with weaker internetwork connectivity.

eFigure 5: Network-based statistic results for and dopamine measures in various regions (Gordon parcellation).

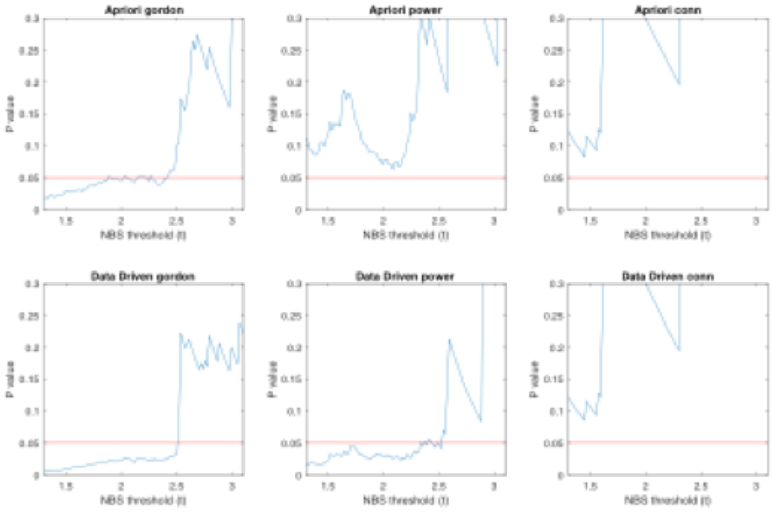


eFigure 6: Network based statistic identifies subnetworks significantly associated with limbic dopamine measures for a range of parcellations

18F-DOPA Salience Network



11C-PHNO Salience Network



Identifying Overlap Between Dopamine-Associated Nodes and Network Hubs

Experiment 1: Dopamine synthesis capacity (^{18}F-DOPA)

We next investigated whether the dopamine-associated nodes identified in the previous step, overlapped significantly with nodes that were classified as information-processing hubs. Within the salience network we found that regardless of how many nodes were defined as hubs within our range of investigation (i.e. the top ranked 10-40%), these nodes were likely to be dopamine-associated nodes, and this overlap was significantly more likely than expected by chance for all hub thresholds (see Figure 4B), and this was the case for all parcellations and methods of node assignment(eFigure 7). The Dice Coefficient between nodes in salience-FDOPA subnetworks and combination hubs across a range of thresholds is shown in Figure 4C, illustrating that the nodes that are most strongly associated with dopamine synthesis capacity (i.e. those surviving the more stringent network-based statistic thresholds) are also the most likely to be key information processing hubs (as defined by resting state functional connectivity).

The Dice Coefficient between combination hubs and the dopamine-associated nodes within the default mode network was numerically greater than the random network at all thresholds but this difference was only statistically significant for certain hub thresholds and parcellation (see figure 4B and eFigure 7).

Experiment 2: Dopamine release capacity (^{11}C-(+)-PHNO)

The Dice Coefficient between combination hubs and the salience-PHNO subnetworks were numerically greater than the mean overlap expected of the random network, but this difference was only statistically significant for certain thresholds and parcellations (figure 4B and eFigure 7).

Overlap between Experiments

Dice overlap scores between the ^{11}C-PHNO and ^{18}F-DOPA associated nodes ranged from 0.36 at the most stringent NBS threshold where equal node networks existed (number of nodes=11), to 0.92 at the least stringent threshold(number of nodes=36). None of these overlaps were greater than would be expected by chance ($p>0.20$ for all thresholds). We then investigated which nodes were in dopamine associated networks at the most stringent threshold, and were also combination hubs (ranked in the top 11/40 nodes in both experiments). Only two nodes fulfilled these criteria, these were located bilaterally in the insula (see figure 4A(iv)).

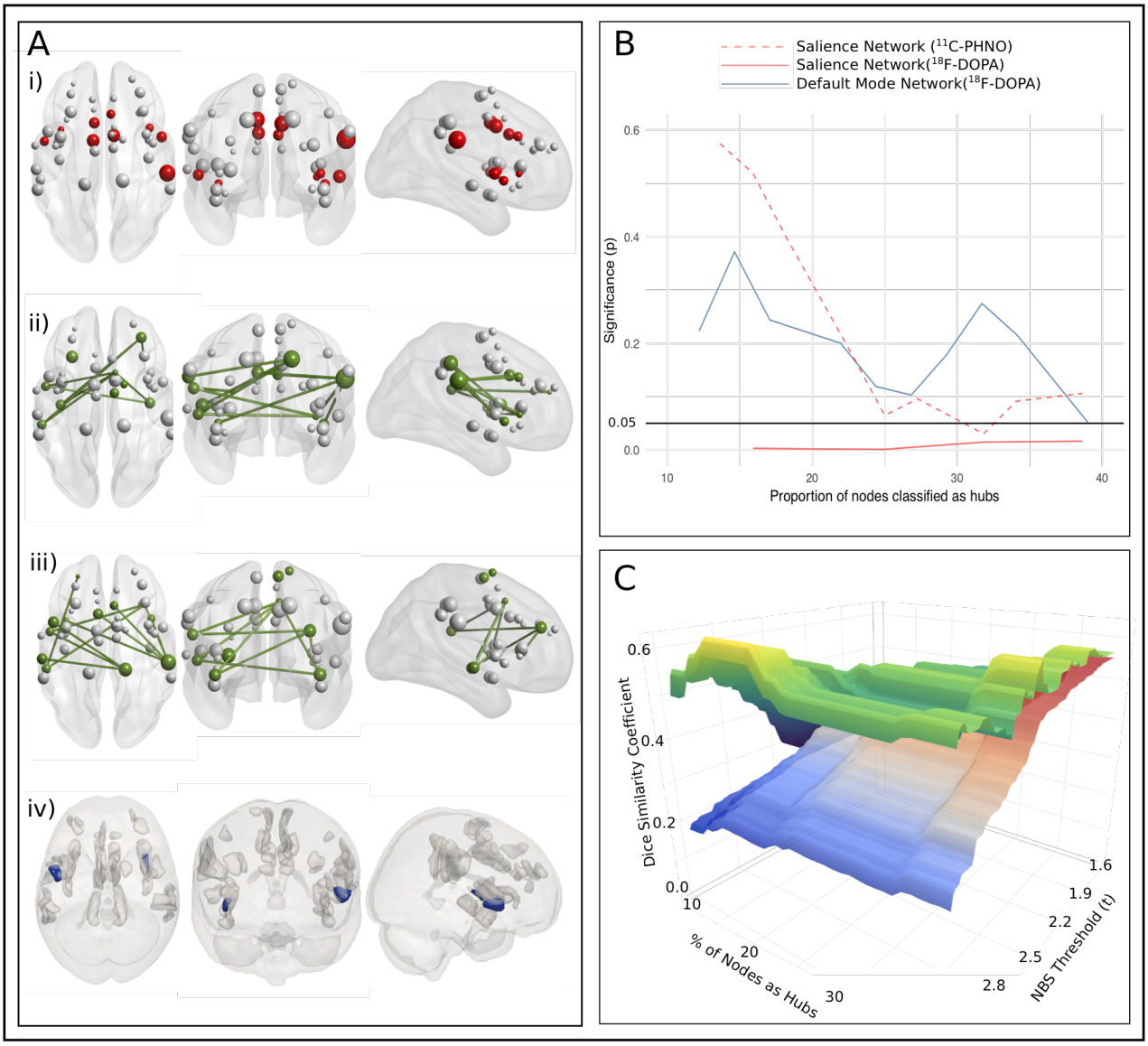


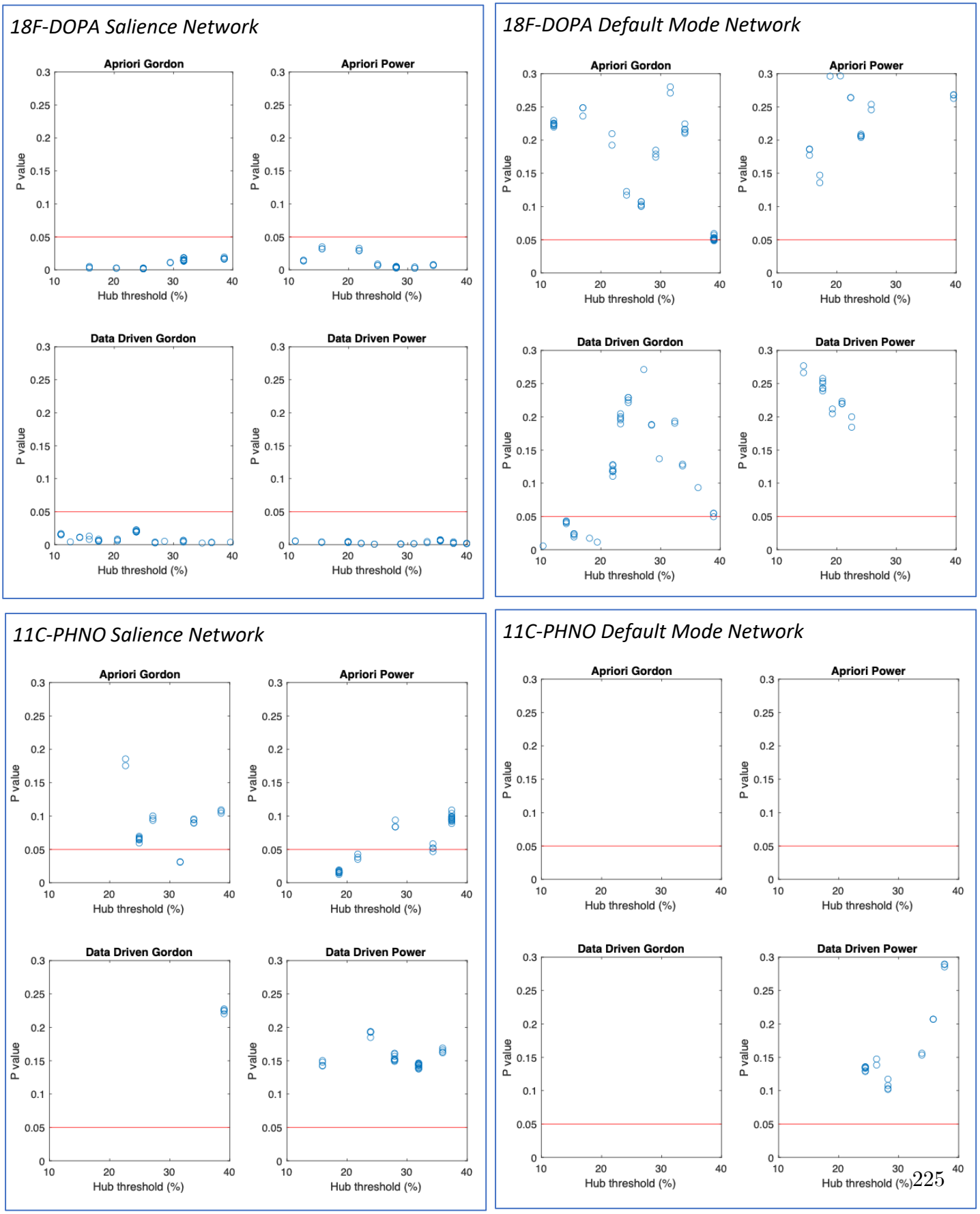
Figure 4: Characterisation of dopamine associated subnetworks

(A) Salience network hubs and dopamine associated subnetworks: Red nodes represent network combination hubs. Green nodes and edges represent dopamine-associated network, in which edge strength correlates with limbic dopamine synthesis capacity. (i) Hub nodes in experiment 1 (ii) dopamine associated network in Experiment 1 at a specific network based statistic threshold (iii) dopamine associated network in Experiment 2 (iv) 2 nodes classified as both dopamine associated nodes and hub nodes in both experiments at the most stringent threshold.

(B) Graph displaying whether overlap between dopamine-associated nodes and combination hubs is significant.

(C) Illustrating the overlap between salience network hub nodes, and nodes involved in ^{18}F-DOPA associated subnetworks. The top (green-yellow) layer represents the Dice Similarity Coefficients for the observed dopamine-associated nodes and the combination hub nodes, while the bottom (blue-red) layer represents the mean overlap coefficients of 10,000 randomised networks. In this figure, the dice coefficient is plotted individually for each network-based statistic threshold (i.e. not averaged as in the construction of 3B)

eFigure 7: Significance of overlap between nodes showing strongest association with limbic dopamine measures and network combination hubs, for a range of parcellations



Discussion

Using rfMRI and a dual-tracer PET paradigm we demonstrate a strong relationship between limbic dopamine function and salience network functional connectivity in humans. Both the salience network and mesolimbic dopamine system are central to the pathophysiology of various neuropsychiatric disorders (4,5,25,26). To our knowledge, however, this is the first human study to both measure limbic dopamine function, and investigate its relationship with the salience network.

Specifically, we demonstrated that stronger connectivity within the salience network was directly associated with limbic dopamine synthesis capacity, and contrary to our initial hypothesis was inversely associated with limbic dopamine release capacity. Furthermore, the biological relevance of this result is supported by the finding that there was significant overlap between nodes in salience subnetworks associated with dopamine synthesis capacity, and nodes separately identified as information processing hubs. We also identified default-mode subnetworks in which edge strength was inversely correlated with synthesis capacity.

The relationship between mesolimbic dopamine function and the salience network

The current study advances our understanding regarding the relationship between mesolimbic dopamine activity and salience network function. Preclinical studies have suggested a link between mesolimbic dopamine function and nodes of the salience network(27–29). However, a precise homologue of the salience network is not present in rodent models, both due to the species-specific nature of cortical networks, and the fact that in humans the network is characterised by the presence of Von Economo neurons, a distinct set of pyramidal neurons, which are not observed in rodents(31,51,52). Previous studies in humans have used rfMRI in combination with pharmacological manipulations of the dopamine system(32,53–55). Without the use of PET, however, it is not possible to obtain a measure of the dopaminergic effect of

the pharmacological intervention, which can vary significantly between individuals for the same dose. Furthermore, drug challenges perturb the system widely, causing various neurochemical changes across the brain and affecting neurovascular coupling(56). In contrast, our resting state data was obtained in a drug free state, and ^{18}F-DOPA PET indexes physiological dopamine function.

Although previous studies have integrated PET and the examination of resting state networks, these have predominantly obtained only measures of baseline dopamine receptor availability(57–60). Two studies have measured dopamine function, but did not examine the relationship with the salience network(34,61).

Dopamine synthesis and release capacity

We hypothesised that release and synthesis capacity would capture similar facets of a single construct – the activity of an individual's mesolimbic dopamine system. Our finding of divergent relationships between these two measures and salience network connectivity does not support this interpretation. Both release and synthesis capacity are complex signals, and the relationship between the two is not clear(62,63). Synthesis capacity represents the rate of L-DOPA decarboxylation, and depends on the number of dopaminergic neurons, and their mean firing rate. Measures of release capacity will be determined by the reactivity of mesolimbic dopamine neurons to the effects of amphetamine. Agonist tracers such as 11C-PHNO preferentially bind to the high affinity state of the D2 receptor(64,65). This means that our measure of percentage release could be affected by the proportion of D2 receptors in a high affinity state as well as the level of dopamine release. Future studies combining antagonist and agonist radiotracers would help determine the potential influence of inter-individual differences in the proportion of D2 receptors in the high affinity state. A tentative hypothesis that unites our findings assumes that, in a healthy individual, dopamine synthesis capacity reflects a summary measure of tonic dopamine neuron firing and appropriate adaptive phasic firing; while release capacity

reflects that individual's propensity for spontaneous phasic firing in the absence of behaviourally relevant stimuli(66). Taken with the finding that reduced salience network connectivity is observed in disorders of aberrant salience processing, this suggests a model in which greater salience network connectivity is associated with the appropriate attribution of salience, mediated by robust adaptive dopaminergic signalling; while a propensity for stimulus independent dopamine neuron firing is associated with weakening of the network, and misattribution of salience(67–71). This is a speculative interpretation, however, and assumes that the consequences of higher dopamine synthesis capacity in healthy participants differ from those in patient populations where it has been linked to disorders of salience(72).

Dopamine Pathways

Preclinical research has often focused upon the dopamine neurons of the ventral tegmental area. The limbic striatum is a major projection target for these neurons, and as such an appropriate region of focus. In rodents, however, the mesolimbic pathway is proportionally larger than in humans, and therefore although the associative striatum receives dopaminergic innervation from the nigra, parts of the human midbrain-associative striatum pathway are homologous to the rodent mesolimbic pathway(73,74). As a result, it is not surprising that the relationship observed between the salience network and limbic dopamine function was also seen when using measures of associative striatum dopamine function. No relationship, however, was seen with dopamine measures obtained from the sensorimotor striatum and salience network connectivity, although an association was seen with release capacity in this region and sensorimotor network connectivity suggesting a degree of functional specificity in the relationship between dopamine measures and network connectivity.

Clinical Implications

Structural and functional abnormalities within the salience network are a common biological substrate of mental illness, and exist trans-diagnostically across a broad range of disorders including depression, schizophrenia, and Parkinson's disease(4,5,75–77). The mesolimbic dopamine system is also affected in these disorders(25,26,78,79). Our finding that the two systems show coupling in humans could explain why they are disordered in several illnesses, as dysfunction in any one of the network's nodes could conceivably lead to impairment across both systems. Our findings also highlight opportunities for the development of pharmacological interventions. The ability to link the effects of quantifiable neurochemical modulation to change in network function, raises the possibility of mapping receptor actions to desired network alterations.

Limitations and Further Questions

Given that the firing of mesolimbic neurons has been shown to provoke widespread neural activity in regions receiving no direct dopaminergic innervation(29), our findings could be interpreted as indicating that mesolimbic dopaminergic signalling is able to regulate salience network function. However, while differences in dopaminergic tone could feasibly shift the balance between the two networks, it is not possible for us to infer the direction of causality.

Likewise, the precise site of relevant dopaminergic activity is not clear. ^{11}C-(+)-PHNO and ^{18}F-DOPA are unable reliably characterise dopamine function outside of the striatum, and it was therefore not possible to test whether a relationship between direct dopaminergic innervation of network nodes and network connectivity also existed.

We used an eyes closed resting state scan, and some networks have shown greater reliability when participants have kept eyes open. The differences are relatively

small, however, and therefore unlikely to have significantly influenced our findings(80).

The use of acute dopaminergic challenges during simultaneous PET-MRI would allow for the study of intra-individual effects of dopaminergic release upon network average strength and organisation, and may help to disentangle some of these issues. Studies in clinical populations, such as individuals with schizophrenia, where measures of both dopaminergic and network function may show wider ranges(81), and the inclusion of behavioural tests would help further determine the relevance of these findings to pathophysiology and psychopathology.

Measures of dopamine function showed strong associations with salience network connectivity, and in the case of dopamine synthesis capacity this was particularly the case for nodes that were identified as information processing hubs within the salience network. These findings are relevant to developing integrated models of brain function in health and disease, and for the development of treatments that attempt to restore network function via neurochemical modulation.

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Conflicts of interest

O.H. has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organised by Astra-Zeneca, Autifony, BMS, Eli Lilly, Heptares, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. Neither Dr Howes or his family have been employed by or have holdings/ a financial stake in any biomedical company. M.M. has consulted for Cambridge Cognition, Lundbeck and Forum Pharmaceuticals in the past 3 years. He has also received research funding from Takeda, Eli Lilly and Roche. The other authors declare no competing financial interests.

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Supplementary Information

Participants

Ethical permission was obtained from the local ethics committee, and all participants provided informed written consent. Healthy controls were recruited via advertisements online and in local media. Subjects had no history of psychiatric or neurological disorders, and had a urine drug screen and pregnancy test (where appropriate) prior to scanning.

PET Data Acquisition and Analysis

Participants were not permitted to smoke or consume caffeine for four hours preceding the scan. After acquiring a CT scan for attenuation correction, PET images were acquired using a Siemens Biograph HiRez XVI PET scanner (Siemens Healthcare, Erlangen, Germany) at Imanova Centre for Imaging Sciences.

Experiment 1: FDOPA study

One hour prior to scanning, participants received 400mg entacapone and 150mg carbidopa, to prevent formation of radiolabelled metabolites and reduce peripheral metabolism. Approximately 160 MBq of ^{18}F-DOPA was administered by bolus intravenous injection. The quantification pipeline was consistent with previous works.[1] Correction for head movement during the scan was performed by denoising the non- attenuation-corrected dynamic images using a level 2, order 64 Battle-Lemarie wavelet filter. Frames were realigned to a single reference frame, acquired 20 minutes post-injection, employing a mutual information algorithm.[2,3] The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images, creating a movement-corrected dynamic image, which was used in the analysis. Realigned frames were then summated to create an individual motion-corrected reference map for the brain tissue segmentation. The cerebellum was used as a reference region, and K_i^{cer} was calculated with the Patlak-

Gjedde graphical approach adapted for reference tissue input function[4]. Image processing and quantification was done using in-house code with Matlab 2012b.

Experiment 2: PHNO study

Approximately 170 MBq of ^{11}C-(+)-PHNO was administered by bolus injection. After the administration of the radiotracer, dynamic emission data were acquired continuously for 90 minutes. The dynamic images were reconstructed using a filtered back-projection algorithm into 31 frames (8 x 15 seconds, 3 x 60 seconds, 5 x 120 seconds, 15 x 300 seconds) with a 128 matrix, a zoom of 2.6 and a transaxial Gaussian filter of 5mm.

An individual parcellation of the brain was implemented in MIAKAT release 4.2.6 (http://www.miakat.org),[5] SPM12 and FSL (version 5.0.9). Cerebellar grey matter was used as the reference region, and the simplified reference tissue model (SRTM) was used to derive BP_{ND} from the regional time activity curves.[6,7] The magnitude of dexamphetamine-induced dopamine release within the limbic striatum was quantified as the percentage change in BP_{ND} in the dexamphetamine condition vs. baseline (no dexamphetamine) condition.

$$\Delta BP_{ND} = 100 \cdot \frac{BP_{ND\,(baseline)} - BP_{ND\,(dexamphetamine)}}{BP_{ND\,(baseline)}}\%$$

MRI data acquisition

Participants were instructed to remain still, keep awake, and keep their eyes closed.

Experiment 1: FDOPA study

MRI data was obtained using a General Electric (Milwaukee, Wisconsin, USA) Signa HDxt 3T magnetic resonance imaging system. Functional imaging consisted of T2* weighted echo planar image slices. 256 volumes were acquired, consisting of 39 interleaved slices (3.5 mm slice thickness, 3.75 mm x 3.75 mm voxel dimensions in plane) with a repetition time (TR) of 2000 ms, echo time(TE) of 30 ms, and a scan time of 8 minutes 32 seconds.

A structural image was obtained using a gradient-echo scan (TR=7.0s, TE=2.8s, flip angle=11°, in plane resolution=1mm x 1mm, slice thickness=1.2mm, 196 slices).

Experiment 2: PHNO study
MRI data was obtained using a Siemens MAGNETOM Verio 3-T magnetic resonance imaging scanner. Functional imaging involved a multiband sequence based on the multiband EPI WIP v012b provided by the University of Minnesota,[8–11] using a multiband acceleration factor of 2. 238 volumes were acquired, consisting of 72 interleaved slices (2mm thickness, and in-plane resolution of 3 x 3 mm), with a TR of 2000 ms, TE of 30 ms and a scan time of 7 minutes 56 seconds.

A structural image was also obtained using a gradient echo scan (TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, 1 mm isotropic voxels, parallel imaging (PI) factor =2, 160 slices).[12]

fMRI preprocessing
Image pre-processing was performed via the CONN toolbox (version 17.b)[13] for Statistical Parametric Mapping software (SPM 12 (6906)). A standard preprocessing pipeline was used consisting of slice timing correction, realignment, and normalisation to MNI space. Images were smoothed with a Gaussian kernel of 8mm full-width-half-maximum. The ART toolbox was used to account for motion and artefact detection using anatomical component based correction (aCompCor) of

temporal confounds relating to head movement and physiological noise. This method models noise effects at a voxel level based on estimates derived from principal components of noise regions of interest (white matter and CSF, eroded by one voxel to minimise partial volume effects), and then removes these from the BOLD timeseries using linear regression. Six residual head motion parameters and their first order temporal derivatives were also entered as regressors into the first level model. A confounding effect accounting for magnetisation stabilisation, and its first order derivative was entered. Artifact/outlier scans (average intensity deviated more than 5 standard deviations from the mean intensity in the session, or composite head movement exceeded 0.9 mm from the previous image) were also regressed out. Preprocessed data were temporally bandpass filtered (0.008-0.09 Hz)

Time-series were extracted from N=333 predefined nodes of interests of the Gordon cortical atlas. The salience and default mode network nodes of the Gordon atlas are displayed in eFigure 1. For each participant, a graph representing a functional connectivity network was constructed, each edge representing the level of functional connectivity between a pair of nodes, which was computed as the z-transformed Pearson's correlation coefficient between their mean time-series.

Graph analysis

MRI-analysis: Atlas selection

The Gordon parcellation is based upon resting state boundary maps observed in a sample of 120 healthy young adults, and shows superior within parcel homogeneity when compared to other parcellations, making it an ideal choice for the analysis of resting state data.[14] In order to demonstrate robustness of our findings, we also undertook all analyses using two alternative atlases - the Power atlas (a collection

of 264 10 mm diameter spheres derived from connectivity data in over 300 healthy volunteers performing various tasks),[15] and the CONN network atlas (a 32 node atlas in which nodes are defined on the basis of an independent components analysis of 497 subjects from the Human Connectome Project).[13]

MRI-analysis: Network Strength

For a network formed of N nodes, the average network strength $\bar{s}$ can be computed similarly to the link density ρ of unweighted networks[16]:

$$\bar{s} = 2/N(N-1)\sum_{i,j=1}^{N} w_{ij}$$

MRI-analysis: Community Detection

On the basis of the original Gordon atlas labels 41 nodes were a priori defined as belonging to the default mode network, and 44 to the cinguloopercular/salience network (referred to in the current paper as the salience network).[14] The Power atlas assigns 32 and 58, while the CONN atlas assigns 7 and 4 nodes to the salience and default mode networks respectively. As a result, the networks of interest were defined across a wide range of scales both in terms of node volume, and maximal network size.

In addition to the apriori network labels, however, we also ran a whole brain community detection algorithm for each atlas,[17] to generate definitions of the salience and default mode networks based on the connectivity patterns present in the current datasets. To accomplish this each subject's fully weighted functional connectome was subjected to the Louvain community detection algorithm, and the results of this were used to generate community assignments at the group level (individual level community assignments are not appropriate for subsequent analyses).[17] Due to the non-deterministic nature of the Louvain algorithm, a previously described consensus clustering approach was employed,[18] negative weights were treated symmetrically,

and the gamma parameter was set to 1.7 as this produced community sizes in relative agreement with existing parcellation schemes.

Identifying Dopamine Associated Nodes– Network Based Statistic

In order to identify whether specific subnetworks show a significant relationship with limbic dopamine synthesis capacity we used the Network-Based Statistic (NBS) to investigate salience, default mode, sensorimotor, and visual networks separately (the method is summarised in Figure 2A in the main text).[19] A t-statistic was generated for each edge based on the Pearson correlation coefficient computed over each population between the functional connectivity z values of that edge, and the limbic striatum K_i^{cer} or ΔBP_{ND} values (i.e. a positive value indicates that greater limbic dopamine synthesis/release capacity is associated with stronger connectivity at that edge). This generated a group level PET-MRI graph for each cohort, which was thresholded at 100 separate thresholds (t=1.3-3.1, equivalent to p=0.2-0.005 for n=23). The connected component with the greatest density (i.e. greatest number of edges) within this t-statistic graph was then determined at each of these t-thresholds. If there were multiple networks of equal density the one with greatest average strength in the weighted version of the graph was selected. Permutation testing was performed to calculate a p-value for each threshold, by determining whether the density of the densest component was significantly greater than expected by chance. This was calculated by comparing the density of the observed component with densest components in 10,000 PET-MRI graphs generated for each threshold (t=1.3-3.1) via random assignment of the K_i^{cer} or ΔBP_{ND} values. We subsequently term the components identified as showing a relationship with limbic dopamine function as 'dopamine-associated subnetworks'. We repeated the analysis using measures for dopamine function in the associative and sensorimotor striatum in place of the limbic measure. We used the same approach to examine inter-network connectivity between salience and default mode networks.

Hub Node Identification

Group level binary graphs were constructed for the salience and default mode networks separately. First individual level graphs were rescaled by subtracting from each individual participant's graph that participant's average network strength, and dividing by the standard deviation of all that network's edges' strength. In this way all participants then had an average network strength of zero and so individuals with greater network mean strength would not have undue topological influence.

We next averaged across individuals to create a group level graph. Proportional thresholding was performed on this group averaged matrix by assigning a value of 1 to all edges with connection strength above a set threshold, and setting all remaining edges to 0. We used 100 thresholds, retaining 20% of edges at the most lenient threshold, and 7% at the most stringent. There is no 'correct' set of thresholds but at more lenient thresholds one risks including a high degree of spurious connections, while at more stringent thresholds the graph became overly fragmented. The fact that this is a more lenient range than reported elsewhere is appropriate given we are investigating intranetwork connectivity, where there will be a lower proportion of spurious edges.[20]

Graph metrics were computed using the Brain Connectivity Toolbox.[21] Node *degree* refers to the number of neighbours a node has, and is thus a measure of the local, direct importance of a node: $k_i = \sum_{j=1}^{N} A_{i,j}$.[22] While this intuitively captures the relative importance of a node within a network, in correlation based graphs, it may also reflect membership of a larger community, as opposed to the importance of the node in information processing.[23] We therefore also calculated for each node *betweenness centrality* B_i,,[22] and the node *participation coefficient* PC_i.[24] The node betweenness centrality measures the proportion of shortest paths between all pairs of nodes that pass through it, and reflects its position as a potential information

broker in the network. It is formally defined as: $B_i = \sum_{m,n=1|i\neq m\neq n}^{N} \frac{|\sigma_{m,n}(i)|}{|\sigma_{m,n}|}$, with $|\sigma_{m,n}|$ the number of shortest paths between nodes m and n.[22] The node participation coefficient was calculated after first assigning each node to a community using the Louvain community detection algorithm.[17] A participation coefficient of zero means that all the edges of a node are restricted to its own community, indicating a rather local role, whereas a value approaching 1 means that its edges are evenly distributed among all the communities of the graph – indicating that the node plays a role in integrating different clusters of the graph. The participation coefficient is defined as $PC_i = 1 - \sum_{C=1}^{N_c} \left(\frac{k_i^C}{k_i}\right)^2$, $PC_i = 1 - \sum_{C=1}^{N_c} \left(\frac{k_i^C}{k_i}\right)^2$, with c the degree of a node restricted to community C.

At each MRI threshold, every node was ranked on each of these metrics, and the mean rank of each node across MRI thresholds was then calculated. We then set a rank threshold, and for each metric selected only the nodes ranking above it. If any node ranked above this threshold for all three metrics it was termed a *combination hub* (main text Figure 2B steps A-C), highlighting its importance as an all-round information processing node. By next lowering the rank threshold we gradually increased the number of nodes meeting combination hub criteria, and so defined sets of combination hubs comprising between 10 and 40% of the total number of nodes. In some cases it is possible a specific hub threshold might have no eligible nodes (e.g. in the main paper figure 4B – the ^{18}F-DOPA salience network does not have combination until the threshold reaches 15%).

Identifying Overlap Between Dopamine Associated Nodes and Network Hubs

After identifying nodes within the default mode and salience networks that showed an association with measures of limbic dopamine function, we sought to identify whether these dopamine associated nodes tended to overlap with nodes classified as combination hubs (as defined above using the rfMRI data).

The overlap of dopamine associated nodes and combination hubs was quantified using the Dice Similarity Coefficient:[25,26]

$$Dice\ Similarity\ Coefficient = \frac{2|A \cap B|}{|A| + |B|}$$

A is the set of nodes in the dopamine associated subnetwork and B is the set of combination hub nodes. The Dice Coefficient was calculated for each of the 100 NBS thresholds (t=1.3-3.1) and then averaged to give a single 'true' score (main textFigure 2B part D). We then randomly selected an assortment of nodes, equal in number to the number of nodes present in the most leniently thresholded original network-based statistic subnetwork (main text Figure 2B part E). Next, we randomly deleted a node from this original assortment whenever the number of nodes in the 'true' subnetwork dropped as NBS threshold stringency increased (main text Figure 2B part F). This gave us 100 thresholds for this randomly generated subnetwork, and for each we calculated the Dice Coefficient with the same combination hubs, and then calculated a single mean 'random' Dice Coefficient as before. We repeated this procedure 10,000 times yielding 10,000 random Dice Coefficients (main text Figure 2B parts G-H), which allowed us to test the significance of the true Dice Coefficient (main text Figure 2B part I). This procedure was then repeated for each of the combination hub thresholds (10-40%), thereby giving a p-value for each hub threshold.

At some more lenient network-based statistic thresholds the network-based statistic defined dopamine associated networks contained all nodes of the SAL/DMN networks. In these cases, all nodes will overlap with the hub nodes, and so it is not valid to test if overlap is statistically significant. In these cases, we increased NBS stringency until the network no longer contained all the nodes in question.

We finally examined overlap between the dopamine associated nodes identified in Experiment 1 and those identified in Experiment 2. In this case we only compared overlap at NBS thresholds where both experiments showed the same number of dopamine associated nodes. We calculated the dice coefficient between the two networks, and compare it to a null distribution generated as before.

Software

Statistical analysis was undertaken in Matlab 2016b and R 3.3.2

Diagrams were constructed in R using ggplot2 (2.2.1) and plotly (4.7.1).

Ball and stick networks diagrams (figure 4A) were constructed using BrainNet viewer.[27]

Node diagram (eFigure1) was constructed using ITK-SNAP[28] and ParaView.[29]

Results

Correlation between rfMRI motion (mean framewise displacement) and network measures

Correlation examined	^{18}F-DOPA	^{11}C-PHNO
Motion:SAL strength	r_p=-0.17, p=0.47	r_p=0.17, p=0.43
Motion:DMN strength	r_p=0.14, p=0.52	r_p=0.03, p=0.89
Motion:(SAL-DMN) strength	r_p=-0.23, p=0.31	r_p=0.09, p=0.67
Motion: Dopamine measure	r_p=0.02, p=0.9	r_p=0.15, p=0.49

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7. Conclusions and future work

Summary of findings

The work presented in this thesis examined striatal function in individuals with psychotic disorders, individuals exposed to risk factors for psychotic disorders, and healthy controls.

The main findings are firstly that dopamine dysfunction in psychosis does not occur uniformly across the striatum but shows significant spatial variability. Furthermore, this variability shows a relationship with the severity of certain psychotic symptoms, dependent on corticostriatal connectivity patterns. I also showed that exposure to environmental risk factors for psychosis led to changes in corticostriatal connectivity, and that these changes were linked to changes in salience processing. Finally, I showed that in healthy controls, striatal dopamine function and salience network connectivity are linked.

Specific findings are as follows:

- Chapter 3: I aimed to synthesise the results of all PET studies that have measured striatal presynaptic dopamine function in schizophrenia, and thereby determine where within the striatum dopaminergic dysfunction is greatest.
  i. *Hypothesis: Presynaptic dopamine function will be greater in individuals with schizophrenia compared to healthy controls.*

     Results: Presynaptic dopamine function was significantly elevated in individuals with schizophrenia relative to controls with a summary effect size of g=0.68

ii. *Hypothesis: This presynaptic hyperdopaminergia will not occur uniformly across the striatum but will be greater in certain subdivisions compared to others.*

Results: Significant differences were found between patients and controls for associative (schizophrenia – g=0.73, p = 0.002) and sensorimotor (schizophrenia – g = 0.54, p=0.009) subdivisions, but not for the limbic subdivision (schizophrenia – g=0.29, p=0.09). In individuals with schizophrenia, the difference between associative and limbic subdivisions was significantly greater in patients compared to controls (g=0.38, P=0.004).

- Chapter 4: I used PET to measure dopamine synthesis capacity in individuals with schizophrenia, and resting state fMRI to parcellate the striatum of these individuals on the basis of corticostriatal connectivity patterns. On the basis of this, to then investigate whether dopamine dysfunction within specific striatal regions is linked to the specific symptoms one would predict on the basis of the connected cortical area.
  i. *Hypothesis: Specific symptoms will be associated with dopamine dysfunction in specific striatal subregions that show preferential connectivity with functionally-relevant cortical regions. I focused on auditory hallucinations and motor symptoms because these are symptoms that have a priori links to well circumscribed (auditory and motor) cortical areas. Specifically, I predicted that both baseline severity and change (following antipsychotic treatment) in severity of hallucinations and of motor symptoms, would correlate with dopamine synthesis capacity in striatal regions preferentially connected to auditory and motor cortex, respectively.*

  Results: I found that the relationship between dopamine function and symptoms varied according to the striatal region examined. Specifically, dopamine function in striatal regions linked to sensorimotor cortex was

associated with both the severity of motor retardation pre-treatment, and the change in motor retardation following treatment with a dopamine antagonist. There was no clear association, however, between hallucination severity and dopamine function within striatal regions linked to the auditory cortex.

ii. *I also undertook an exploratory analysis investigating whether appeared notable relationships between dopamine dysfunction appeared in other cortical connectivity defined striatal subregions and other symptom clusters.*
Results: Dopamine function within the striatal regions linked to the default mode network, and cingulopercular network were associated with negative/cognitive and affective symptoms respectively.

iii. *Finally, I compared our connectivity defined striatal parcellation with published atlas-defined subdivisions, in order to examine whether a individualised data-driven connectivity-based method is able to provide additional information over an atlas-based approach.*
Results: I demonstrated significantly greater orthogonality in our individualised connectivity-based approach, which allowed, for the first time to my knowledge, specific subregion-symptom relationship to be investigated.

- Chapter 5: To investigate the cognitive and neurobiological correlates of exposure to chronic psychosocial stressors that are established environmental risk factors for psychosis. To do this I employed resting state MRI and a behavioural task (the salience attribution task) to test the following hypotheses.

  i. *Hypothesis: Individuals with a history of high exposure to chronic psychosocial stressors will display increased aberrant, and reduced adaptive, salience scores compared to individuals with a history of low exposure.*
  Results: In the current study, I demonstrated reduced adaptive salience in individuals that had been exposed to chronic psychosocial stressors. I also found increased scores on the aberrant salience inventory in the exposed

group, but contrary to our initial hypotheses did not detect any between group differences on the aberrant or implicit measures of the SAT.

ii. *Hypothesis: Individuals with a history of high exposure to chronic psychosocial stressors will display altered corticostriatal functional connectivity compared to individuals with a history of low exposure.*
Results: The exposed group displayed increased functional connectivity between the ventral striatum and several cortical regions. A number of these clusters overlapped with cortical areas that make up the cingulo-opercular or salience network.

iii. *Hypothesis: Alteration in corticostriatal connectivity will be related to alterations in salience processing.*
Results: Reduced adaptive salience score were related to increased connectivity between striatal seeds and cortical regions involved in salience processing.

- Chapter 6: To examine the relationship between two key salience processing systems: the cortical salience network and the mesolimbic dopamine system. We used resting state fMRI to characterise the salience network in two separate cohorts – one that had received an ^{18}F-DOPA PET scan (to measure dopamine synthesis capacity), and the other that had received an ^{11}C-PHNO scan before and after amphetamine administration (to measure dopamine release capacity).

  i. *Hypothesis: My primary hypothesis was that individuals with greater striatal dopamine synthesis and release capacity would show greater connectivity within the salience network, and, because of the reciprocal relationship between salience and default mode networks, weaker connectivity within the default mode network.*
  Results: I demonstrated that stronger connectivity within the salience network was directly associated with limbic dopamine synthesis capacity,

and contrary to our initial hypothesis was inversely associated with limbic dopamine release capacity. I also identified default-mode subnetworks in which edge strength was inversely correlated with synthesis capacity.

ii. *Hypothesis: I hypothesized that there would not be a uniform association between dopamine function and connectivity but that hub nodes would show the strongest association with dopamine.*
Results: Significant overlap existed between nodes in salience subnetworks associated with dopamine synthesis capacity, and nodes separately identified as information processing hubs.

In summary, I demonstrated in Chapter 3 how dopamine dysfunction is most pronounced in the dorsal as opposed to limbic striatum, suggesting that the mesolimbic hypothesis of schizophrenia is unlikely to be accurate. This was built upon in chapter 4 to show that different symptoms vary in their association with dopamine function across the striatum, depending on the functional connections between striatal and cortical regions. While, I have at times highlighted the role of the dorsal striatum, it is however likely that the limbic striatum still plays an important role in the pathophysiology of schizophrenia. It was the limbic striatum that showed changes in connectivity related to environmental exposures and salience processing measures in chapter 5. It was also dopamine function within the limbic striatum that showed a strong relationship with salience network connectivity in chapter 6.

Limitations

Specific limitations have been covered in each chapter. Below I discuss a more general limitation of functional connectivity studies given that this was not previously discussed.

A frequent aim of science is to reveal causal structures, and neuroscience is no exception. Although correlational data has value in and of itself, for example in classification tasks, in neuroimaging experiments we frequently wish to understand information processing pathways within the brain, and the influence that various brain regions have upon one another.

The inferences we are able to draw in standard functional connectivity experiments, however, are limited, due to the potential confounding inherent in the vast number of unobserved variables potentially underlying the observed data. The recorded signals are a tiny fraction of the true causal variables. It is because of this high ratio of the unobserved:observed that methods aimed at imputing *effective connectivity* cannot return causal information[1,2]. There is a potential solution, however, to this quagmire of unlimited confounding. Paradigms involving a perturbation of the system, allow one to step beyond solely correlational experiments. Pharmacological challenges, and noninvasive brain stimulation techniques are examples that may be employed in humans, while preclinical experiments may also make use of optogenetic and chemogenetic techniques.

Future work

The results of the current work present a number of potential avenues for further investigation.

Spatially specific modulation of striatal dopamine function

In Chapter 3 I found that striatal hyperdopaminergia in schizophrenia appears to primarily be located within the dorsal striatum. This raises the possibility that

targeting dorsal striatal dopaminergic neurotransmission specifically, may have benefits in terms of efficacy and side effect burden.

An initial step is to determine whether this is possible. Preclinical studies have demonstrated that M4 positive allosteric modulators act on striatal medium spiny neurons to specifically inhibit dorsal striatum dopamine[3,4]. A PET study using a D2/3 receptor ligand such as ^{11}C-PHNO in combination with an amphetamine challenge could test whether these compounds are able to specifically inhibit dorsal striatal dopamine release in humans.

Dopamine and symptoms in psychosis

In Chapter 4 I used an fMRI informed parcellation of the striatum to investigate the relationship between striatal dopamine function and symptoms in individuals with a first episode psychosis. It would be interesting to compare this approach with a striatal parcellation based on white matter pathways as quantified using diffusion weighted imaging.

It would also be of interest to have a more in-depth phenotypic assessment of patients, for example including tasks and behavioural modelling in order to allow for a more precise quantification of symptom-dopamine function relationships.

Studies of environmental risk factors

In Chapter 5 I examined the effect of multiple environmental factors upon resting state functional connectivity. As discussed, this approach is in contrast to the majority of studies which have tended to focus upon single risk factors. The sample size and distribution of risk factors did not allow for the detection of interaction effects. Determining whether risk factors interact in an additive or synergistic manner is of considerable interest but can be properly investigated only with samples sizes that are orders of magnitude greater than those reported in most

neuroimaging studies. In recent years, the availability of publicly available large-scale datasets such as the UK biobank which in includes neuroimaging measures in addition to genetic, and environmental information allows for testing of these hypothesis with adequate power [5].

Neurochemically informed models of brain networks

In chapter 6 I investigated associations between PET measures of dopamine function and the architecture of resting state brain networks. My findings, however, solely related to *between individual* correlations and do not allow comment on *within individual* relationships between dopamine and functional brain networks. The use of acute dopaminergic challenges during simultaneous PET-MRI would allow for the study of intra-individual effects of dopaminergic release upon network strength and organisation. Furthermore, although generative models of resting state functional connectivity allow one to impute underlying neuronal parameters that could potentially give rise to observed functional connectivity patterns,[6,7] as discussed above, true causal inferences are not possible given the potential for unobserved confounding. The integration of resting state data with simultaneously obtained measures of neurochemical perturbation has, however, the potential to provide information that addresses these issues.

Other areas for further work include studies in clinical populations, such as individuals with schizophrenia, where measures of both dopaminergic and network function may show wider ranges, and the inclusion of behavioural tasks would help further determine the relevance of these findings to pathophysiology and psychopathology. Investigating the relationship between brain networks and other measures of neurochemical function (e.g. ^{1}H-MRS to measure glutamate concentrations) would also be of interest.

I investigated *static* connectivity where for each individual the entire run is condensed into a single functional connectivity matrix. There have been recent advances studying *time varying* functional connectivity in order to take account of the fact that brain states within individuals are not static but show frequent transitions[8]. It would be of significant interest to see the relationship between measures of dopamine function and time varying functional connectivity.

Conclusions

In the current thesis I have shown that dopamine dysfunction in schizophrenia is greatest in the associative striatum, refuting the mesolimbic hypothesis. I have then highlighted the clinical relevance of this by demonstrating how spatial variability in dopamine function may shape psychotic symptoms. I next investigated the potential role of the striatum in the pathogenesis of psychosis by examining how environmental risk factors are associated with altered striatal connectivity and changes in salience processing. Finally, I have shown how striatal dopamine function and the functional structure of the salience network appear tightly linked.

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Appendix A: Work published during thesis

McCutcheon RA, Abi-Dargham A Howes O (2019) Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms *Trends Neurosci* (1-12)

McCutcheon RA, Bloomfield M, Dahoun T, Mehta M, Howes O (2019) Chronic psychosocial stressors are associated with alterations in salience processing and corticostriatal connectivity *Schizophr Res* (1-10)

McCutcheon RA, Nour MM, Dahoun T et al. (2019) Mesolimbic Dopamine Function is Related to Salience Network Connectivity: An Integrative PET and MR Study *Biol Psychiatry* (1-11)

McCutcheon RA, Beck K, Jauhar S, Howes O (2018) Defining the Locus of Dopaminergic Dysfunction in Schizophrenia: A Meta-analysis and Test of the Mesolimbic Hypothesis *Schizophr Bull.* (1-8)

McCutcheon RA, Bloomfield M, Dahoun T, Quinlan M, Terbeck S, Mehta M, Howes O (2018) Amygdala reactivity in ethnic minorities and its relationship to the social environment: an fMRI study *Psychol Med.* (1-8)

McCutcheon RA, Beck K, D'Ambrosio E et al. (2018) Antipsychotic plasma levels in the assessment of poor treatment response in schizophrenia *Acta Psychiatr. Scand.* 137(1) 39-46

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McCutcheon RA, Karr S, Howes O. Drugs Used to Treat Psychosis. (2018) In Haddad P and Nutt D (Eds.) *Seminars in Clinical Psychopharmacology (3rd ed)* London: RCPsych *(in press)*

Pillinger T, Rogdaki M, **McCutcheon RA,** Hathway P, Egerton A, Howes OD (2019) Altered glutamatergic response and functional connectivity in treatment resistant schizophrenia: the effect of riluzole and therapeutic implications *Psychopharmacology (1-13)*

Jauhar S*, **McCutcheon RA*,** Borgan F et al. (2018) The relationship between cortical glutamate and striatal dopamine function in psychosis: a multi-modal PET and MRS imaging study in first episode psychosis *Lancet Psychiatry*

Pillinger T, D'Ambrosio E, **McCutcheon RA,** Howes O (2018) Is psychosis a multi-system disorder? A meta-review of central nervous system, immune, cardiometabolic, and endocrine alterations in first episode psychosis and perspective on potential models *Mol. Psychiatry*

Nour MM, **McCutcheon RA**, Howes OD. (2018) The relationship between dopamine synthesis capacity and release: implications for psychosis. *Neuropsychopharmacology.* 43(6) 1195-1196 Letter to Editor.

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Rogdaki M, Jauhar S, **McCutcheon RA,** Howes O (2016) Treatment-Resistant Schizophrenia in a Patient With 17q12 Duplication *Biological Psychiatry* 80(4) e19-e20

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Appendix B: Additional Analyses

Following the suggestion of the PhD examiners the following analysis was performed on the data presented in chapter 3.

To determine whether patient-control effect sizes differ between subdivisions a multivariate meta analytic approach is required given the lack of independence between subdivision effect sizes. We used an unstructured variance-covariance matrix, and employed the R packages metafor and clubSandwich.

As in earlier analyses, because within-study correlations between outcomes are not reported in all studies, we employed a correlation coefficient of 0.72 based on the lower limit of between-subdivision correlations observed in a set of individual participant data. Another suggested approach is to estimate this coefficient based on the correlation between the available effect estimates in the studies that provide data on both outcomes, and we also used this approach [4].

We found that both the associative ($z=2.4$, $p=0.016$) and sensorimotor ($z=2.1$, $p=0.034$) striatum showed greater patient-control differences in measures of dopamine function compared to the limbic striatum. The comparison between associative and sensorimotor striatum was not significant ($z=1.7$, $p=0.10$) using the original correlation coefficient. However, when using either the coefficient for associative-sensorimotor correlations in individual patient data ($r=0.87$), or that derived from across study correlation ($r=0.97$), the difference was statistically significant ($z = 2.2$, $p=0.03$; $z=2.6$, $p=0.01$ respectively).

This complements our published analysis by showing that the magnitude of patient-control differences differs between subdivisions.